Novel Surface Engineering of Carrier Particles for Dry Powder Inhalation Formulations

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Novel Surface Engineering of Carrier Particles for Dry Powder Inhalation Formulations

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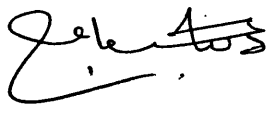

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To

Dr. Siham Abbas

and

Dr. Abdel Raouf El Sabawi

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In the name of God, the most gracious, the most merciful.

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Scientific Publications

El-Sabawi, D., Price, R., Edge, S., Young, P.M. (2005). Novel temperature controlled surface dissolution of excipient particles for carrier based dry powder inhaler formulations. *Drug Development and Industrial Pharmacy.* Accepted.

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Novel Surface Engineering of Carrier Particles for Dry Powder Inhalation Formulations

Dina El-Sabawi

Abstract

Dry powder inhalation formulations typically involve the co-processing of an active pharmaceutical ingredient and a relatively coarse excipient, commonly known as carrier particles, to ensure accurate dose metering, increased device clearance and the dispersion of the respirable particulates upon device actuation.

It is well recognised that there is a critical relationship between the surface characteristics of the carrier particles and the overall delivery performance of a DPI formulation. Such variations in the physicochemical properties of the carrier particles have a direct influence on the de-aggregation behaviour of respirable drug particles, and typically lead to considerable batch-to-batch variations. These uncontrolled modifications threaten the overall efficiency and reproducibility of the inhalation therapy. The uncontrollable nature of the surface properties of commercial grade α-lactose monohydrate is a consequence of the rigorous industrial processing techniques utilised in the preparation of the excipient particles. To overcome these issues, a novel temperature controlled surface etching process has been developed in this study to controllably modify the physicochemical properties of commercial lactose particles. *In vitro* aerosolisation behaviour of surface etched lactose was investigated using salbutamol sulphate as a model drug. Significant difference ($p<0.05$) in the fine particle delivery of the drug was measured upon decreasing the surface roughness of lactose carrier particles. The study further highlighted that the aerosolisation efficiency was highly dependent on the degree of surface etching suggesting the importance and the need to

control and optimise the degree of surface roughness of carrier particles for DPI formulations.

Further work focussed on studying the potential of the surface etched lactose, produced by the temperature controlled surface etching process, as a carrier of choice for inhalation systems aimed for low dose drug delivery. The study indicated that the surface smoothing of lactose particles resulted in a significant increase in the aerosolisation performance at lower drug concentrations (minimum FPF at drug load of 63.5 µg for surface etched lactose compared to a drug load of 135 µg for commercial grade lactose), as a consequence of considerably reducing the presence of the so-called 'active sites'.

Of equal importance to modifying the surface properties of excipient particles for improved aerosolisation performance, is the physicochemical stability of the formulation upon storage at elevated temperature and relative humidity conditions. *In vitro* aerosolisation behaviour of the controlled surface etched lactose and commercial grade lactose formulations was investigated as a function of storage for one and three months at 25°C, 75% RH and one month at 40°C, 75%RH. No significant difference in the delivery performance was observed for the surface etched lactose formulation before and after storage. Accelerated stability testing and *in vitro* investigations of commercial and secondary processed lactose indicated that controlled surface etched lactose maintained drug deposition efficiency with respect to commercial, untreated lactose based formulations at elevated conditions of humidity and temperature.

This study emphasised on the importance of utilising a secondary post-treatment procedure to the bulk crystallisation process of excipient particles for DPI formulations. Improved *inter* and *intra* physicochemical characteristics of excipient particles may significantly enhance the uniformity and reproducibility of *in vitro* and *in vivo* performance of an active pharmaceutical ingredient.

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List of Abbreviations

$\bar{x}$	arithmetic mean
%RH	relative humidity
ΔC_T	difference in solubility
ΔT	difference in temperature
ΔU	potential difference
Å	Angstrom
A	Hamaker's constant (Equations 1.1, 1.2); area (Equations 1.9, 1.10).
AJS	air jet sieved
ANOVA	analysis of variance
BET	Brunauer Emmett Teller
BP	British Pharmacopoeia
C	BET constant (Equation 2.1).
CCI	Carr's compressibility index
CFC	Chlorofluorocarbons
COPD	Chronic obstructive pulmonary disease
C_S	solubility at saturation temperature
C_T	solubility at etching temperature
d	diameter (Equations 1.1, 1.2); distance (Equations 1.3, 1.4, 1.5, 1.6); interatomic spacing (Equation 2.4).
d_{50}	50^{th} of particle diameter
dm/dt	change in mass in relation to time
DPI	Dry powder inhaler
DVS	Dynamic vapour sorption
ED	emitted dose
F	capillary force

F_{el} , F_c, F_w	electrostatic force
FPD	fine particle dose
FPF	fine particle fraction
F_{vdw}	Van der Waals force
HFA	Hydrofluoroalkanes
HPLC	High performance liquid chromatography
LALLS	Low angle laser light scattering
LD	loaded dose
M	mass
MMAD	mass median aerodynamic diameter
MSLI	Multi-stage liquid impinger
N_A	Avogadro's constant
P	relative partial pressure
pMDI	Pressurised metered-dose inhaler
P_o	saturation pressure
q	electrical charge
r	distance (Equations 1.1, 1.2); radius (Equations1.4, 1.6, 1.7, 1.8).
R^2	coefficient of determination
RSD	relative standard deviation
S(T)	solubility at specific temperature
sa	surface area
SD	standard deviation
SEM	Scanning electron microscopy
T	temperature
TST	Twin-stage impinger
V_a	total mass of gas
V_L	volume of saturated solution
V_m	mass of gas in monolayer
W_a	work of adhesion
W_c	work of cohesion
XRPD	X-ray powder diffraction
α	contact angle, anomeric form of lactose
β	contact angle, anomeric form of lactose

γ	surface tension
γ^C, γ^D, γ^{CD}	free energy per unit surface area
ε	permittivity
θ	diffraction angle
λ	wavelength
ρ_b	bulk density
ρ_t	tapped density
σ	cross sectional area (Equation 2.3); undersaturation (Equation 3.1).

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Chapter 1

Introduction

1.1 General introduction

The majority of pharmaceutical formulations are primarily designed to deliver therapeutic medicaments in a solid dosage form preparation *via* a number of routes which can be classified with the following categories: oral, rectal and parenteral routes. The latter includes intravenous, intramuscular and subcutaneous drug delivery of injections which may contain a suspension of solid drug particulates (Byrn, 1982). It is only recently, however, that the respiratory system has been genuinely regarded as a potential port of entry for delivery of therapeutic agents to the systemic circulation (Wall, 1995; Johnson, 1997; Mobley and Hochhaus, 2001). Drug delivery to the respiratory tract has become a well recognised and increasingly effective route for providing therapy to a variety of pulmonary disorders (Hickey *et al.*, 1994). Bronchodilators, steroids, mast cell stabilisers and anti-cholinergic drugs are examples of pharmacologically active ingredients that provide therapeutic treatment for a number of respiratory illnesses such as asthma, chronic obstructive pulmonary disease (COPD), bronchitis and cystic fibrosis (Pritchard, 2001).

Inhalation therapy has been shown to exhibit significant advantages in comparison to other routes. These include rapid onset of action due to local delivery of active ingredients to the bronchi and bronchioles. Furthermore,

lung drug delivery allows the administration of relatively low doses of the active pharmaceutical ingredient having equivalent therapeutic efficacy to solid dosage formulations, while minimising possible side effects associated with the oral route (Timsina *et al.*, 1994). For systemic drugs, the rapid absorption *via* the respiratory bronchioles and alveoli circumvents the degradation of the drug *via* hepatic first pass metabolism as associated with the oral route (Byron and Patton, 1994; Wall, 1995).

The human lung is the organ responsible for the exchange of gaseous substances between the atmosphere and the blood capillaries of the circulatory system. The structural and functional features of the peripheral regions of the lung provide a tremendous surface area of about 100 m^2 for gaseous exchange. Together with the rich supply of blood, the alveoli and terminal bronchioles provide unique characteristics for rapid drug absorption and, thus, high bioavailability (Byron and Patton, 1994; Patton, 1996).

The leading airway into the lung is the trachea which continually bifurcates into a series of bronchi and bronchioles. These areas are described as the conducting airways. The peripheral regions of the lung are associated with the respiratory bronchioles and alveoli which are the basic structure responsible for the blood capillary gas exchange (Patton, 1996). A schematic diagram of the structure of the human lungs is shown in Figure 1.1.

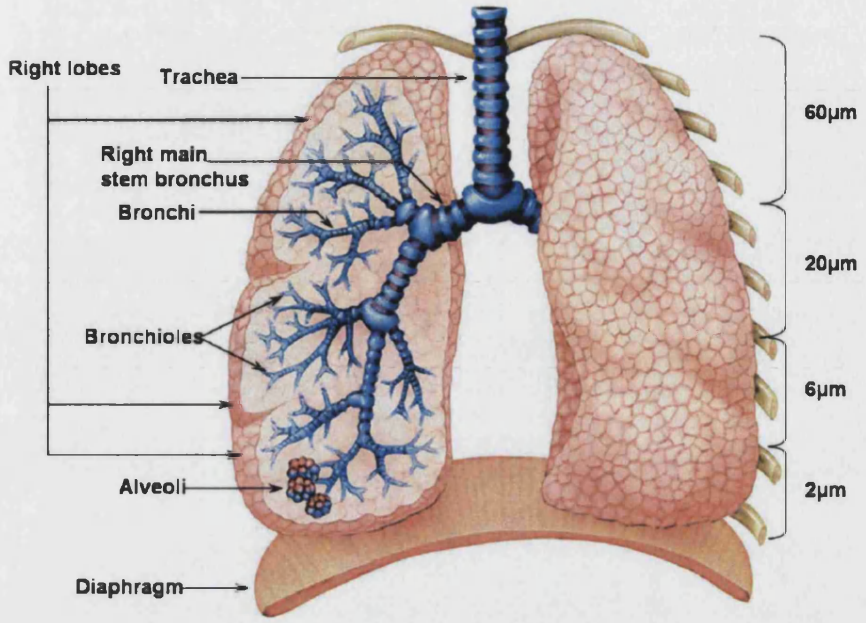

Figure 1.1. Description of the structure of the human lung.

The delivery of a therapeutic aerosol dose to the respiratory tract is currently possible *via* three systems: pressurised metered-dose inhaler (pMDI), dry powder inhaler (DPI) and a nebuliser (Borgström and Newman, 1993; Clark, 1995a; Pitcairn *et al.*, 1995; Concessio *et al.*, 1997; Pitcairn *et al.*, 2000). The characteristic physicochemical properties of the formulations and the engineering of these inhalation devices have been shown to greatly influence the efficiency and therapeutic efficacy of inhalation therapy (de Boer *et al.*, 1995; Steckel and Müller, 1997a; Srichana *et al.*, 1998a; Zeng *et al.*, 2002a; Zeng *et al.*, 2002b; Labiris and Dolovich, 2003).

Until very recently, the mainline treatment for inhalation therapy was *via* pressurised MDI delivery systems, which typically consisted of an active pharmaceutical ingredient suspended in a suitable chlorofluorocarbon (CFC) propellant system. However, its long term future has been put into question due to several disadvantages (Timsina *et al.*, 1994). These include the deleterious effects on the ozone layer and related global warming issues arising from the use of CFCs, inter-patient variability resulting from poor

actuation-inspiration co-ordination and certain limitations surrounding the metering system within a pMDI, which limits the delivered dose to 1 mg (Ganderton and Kassem, 1992). Furthermore, the relatively high velocity of the delivered droplets often results in considerable drug loss due to impaction in the oropharynx (Brambilla *et al.*, 1999). In an attempt to overcome the drawbacks associated with the use of the environmentally harmful CFCs in pMDI systems, alternative non-CFC propellants were developed. These are the hydrofluoroalkanes (HFAs). However, such conversion from CFCs to HFAs was found to be problematic to some extent due to variations in their physicochemical properties, such as polarity, vapour pressure and density (Crowder *et al.*, 2001). Moreover, although less than CFCs, HFAs were also found to contribute to global warming.

Nebulisers do not utilise volatile propellants. With the use of a compressor, a liquid aerosol of drug is nebulised as a mist. Nebulisers are more commonly utilised in a hospital setting for patients suffering from severe respiratory distress requiring high doses of medicament. Their lack of portability, high cost and low therapeutic efficacy has limited their wider use (Horsley, 1988).

To overcome some of the issues relating to the use of pMDIs and nebulisers, the introduction of dry powder inhaler (DPI) device in the 1970s has offered several advantages over the other systems (Bell *et al.*, 1971). In a dry powder inhaler formulation, the drug is delivered as a powder without the need of a propellant, avoiding the related environmental concerns encountered with pressurised metered dose inhalers (pMDIs). Dry powder inhaler devices are portable and are automatically breath actuated, which removes the requirement of the need to synchronise actuation of the device with the inhalation manoeuvre of the patient (Timsina *et al.*, 1994; Ashurst *et al.*, 2000).

To deliver the therapeutic dose to either the conducting or peripheral regions of the respiratory tract, the active pharmaceutical ingredients need to be formulated in the optimum particle size range for efficient delivery (Lord and Staniforth, 1996). For treatment of respiratory diseases, such as asthma and

COPD, the active ingredients need to be delivered to the smooth muscles of the conducting airways. These require the formation of particles in the size range of 2-7 μm. For systemic delivery, however, particles need to be delivered in the 0.5-2 μm size range (Hinds, 1999). Efficient delivery to peripheral conducting airways is markedly influenced by the physicochemical properties of the aerosol particles together with certain physiological considerations that ought to be encountered. For example, in part of the structural design of the respiratory tract, the oropharynx exhibits a 90° bend which efficiently collects air-suspended particulates *via* impaction (Li and Edwards, 1997). Furthermore, inhaled particles have to circumvent a tortuous path that is a resultant of concomitant branching and narrowing of the airways. Equally important is also the mucociliary clearance mechanisms and high atmospheric humidity that may potentially inhibit inhaled particles from reaching peripheral regions of the lung (Malcolmson and Embleton, 1998). Thus, both the site and the mechanism of deposition of drug particulates in the lung can be to some extent influenced by the particle size of air suspended particulates.

In an attempt to meet these stringent requirements, the active drug in a DPI formulation needs to be micronised within a critical particle size range. This high-energy process usually leads to highly cohesive powders and poor flowability. To increase flowability and aid in the metering and deaggregation of the therapeutic dose, the micronised powder is typically blended with coarse carrier particles (Staniforth, 1980; Ganderton, 1992). The carrier particles tend to be of sufficient size to allow adequate fluidisation of the formulation out of the device. These particles subsequently, deposit in the oropharynx. Upon actuation of a dry powder inhaler device, the forced inspiration breath of the patient provides sufficient energy to both fluidise the drug-carrier blend and to dissociate and disperse drug from the surface of the carrier particles (Kulvanich and Stewart, 1987; Ganderton, 1992; French *et al.*, 1996).

The deposition of these re-dispersed particles within the respiratory tract occurs through various mechanisms. For larger particulates (>10 μm), the

main mechanism responsible for deposition in the mouth, throat and upper airways is *via* inertial impaction. Deposition within the conducting airways is dominated by gravitational sedimentation due to the decreased velocity and larger relaxation times for the inhaled particles in the lower bronchi and bronchioles. Peripheral and alveoli deposition is influenced by diffusion and gravitational sedimentation, and is usually exhibited by particles with a diameter in the size range of 0.5 - 2 μm. Particles exhibiting a diameter of less than 0.5 μm are most likely to be exhaled, due to their low mass which limits the influence of gravitational forces and the limited period for diffusion within the peripheral regions of the lung (Malcolmson and Embleton, 1998). Other possible deposition mechanisms include interception and electrostatic attraction (Hinds, 1999).

Considering the critical importance of particle size on the delivery efficiency of a therapeutic dose, respirable sized particles are defined in terms of their aerodynamic diameter. Since the majority of aerosol particles are irregular in shape, with considerable variations in size and density, the influence of their physical properties on aerosolisation behaviour can be accounted for by determining the aerodynamic behaviour of the particles rather than their geometric shapes and sizes. The aerodynamic diameter is defined as the diameter of the spherical particle with a density of 1 g/cm^3 that has the same settling velocity as the particle being investigated (Hinds, 1999).

Over the last two decades, great prominence has been directed towards the research and development of a strategic and structural design for both formulation and device, which would yield efficient and reproducible drug delivery *via* the respiratory tract. However, despite the characteristic properties of the formulation and/or design of the inhalation device, the performance is principally related to the shear forces and turbulent behaviour of the airflow generated from an optimised inspiratory action by the patient (Ziskind *et al.*, 1995). The potential of achieving optimum dispersion and deposition is therefore governed by the co-interaction of a number of key determinants (Concessio *et al.*, 1999; Chew *et al.*, 2000; Clarke *et al.*, 2000), most notably the patient inspiratory manoeuvre. The inspiration technique

critically relies on the synergy between the engineered DPI device and the inspiratory energy created by the patient through the device (Ganderton and Kassem, 1992; Clark, 1995a; Hindle and Byron, 1995; Srichana *et al.*, 1998a).

In spite of the striking advances that have been accomplished lately in the development of pharmaceutical powder formulations, certain aspects related to the upholding of an optimum therapeutic dose delivered to target sites are yet to be embarked upon. Concurrent investigations are being undertaken to further elucidate the rather ambiguous fundamental behaviour of particulate interactions and the role they may exert upon powder behaviour during processing, handling, fluidisation and aerosolisation of formulations.

1.2 Inter-particulate interactions

The importance of inter-particulate interactions in pharmaceutical solid dosage forms cannot be underestimated. Inter-particulate forces influence many bulk properties of powders. These include flowability, mixing, de-aggregation, dispersion, compression and even drug dissolution (Staniforth *et al.*, 1981; Staniforth and Rees, 1982; Staniforth, 1994; Podczeck, 1998a; Clarke *et al.*, 2002).

Particle-particle interactions, with the exception of covalent bonds, occur as a result of long-range physical attractive forces. A covalent bond, is defined as the chemical bond resulting from the attractive forces existing between atoms in a molecule. Such covalent forces are extremely strong exhibiting a typical energy of formation in the range of 300 to 700 $kJ.mol^{-1}$. Particle interactions, on the other hand, are due to significantly weaker physical bonding, usually exhibiting energies of bonding of less than 40 $kJ.mol^{-1}$. Although these forces are much weaker than chemical bonds, their influence however, extends over greater distances, thus, termed as "long-range forces".

Particulate interactions can be broadly differentiated into two main classes, depending on the nature of the two interacting materials. These are namely cohesion and adhesion. Cohesion refers to the interactive forces that exist between particles of the same material, while adhesion refers to the interactive forces that exist between particles of different materials. A powder is said to be adhesive when the component particles tend to readily adhere to the surface of an object of larger dimensions. That is to say, the magnitude of the interaction forces applying between the particle and the surface exceed the magnitude of the separation forces exerted on the particle itself. However, such interactions only exist if one of the interacting parties exhibits a particle dimension of less than 10 μm (Visser, 1995), where gravitational forces play a negligible role in the behaviour of particles with respect to the physical forces.

1.2.1 Forces contributing to particle-particle interactions

Particulate interactions dominating the behaviour of a powder system are governed by a number of interacting forces. These interactive forces are a resultant of the integrated influence of the omni-present van der Waals forces, the dynamic electrostatic (Coulombic) force and capillary force (Visser, 1989).

The extent of such forces is strongly subjective to the influence of various key elements which mainly include particle physical properties such as particle size, shape and surface texture (Podczeck, 1998a; Walz and Sun, 2002) and the surrounding environmental conditions such as temperature and RH (Coelho and Harnby, 1978). The dynamic electrostatic forces, for example, arise from the contact and separation of two different contiguous surfaces during powder handling. Such surfaces may become charged as a result of triboelectrification (Carter *et al.*, 1992; Byron *et al.*, 1997). On the other hand, capillary forces dominate particulate interactions due to the increased risk of the presence of a physisorbed water layer with increasing levels of relative

humidity (%RH) (Price *et al.*, 2000; Price *et al.*, 2002a). A further phyico-mechanical force which needs to be taken into account in DPI based interactions, especially when dealing with irregularly shaped pharmaceutical solids exhibiting rough surfaces, is mechanical interlocking. Mechanical interlocking between particles occurs when particles interlock as they come into contact, and may be a significant contributor to the overall particulate interactions (Zeng *et al.*, 2001a). A more detailed overview of each of these physical forces is described below.

1.2.1.1 Van der Waals forces

The van der Waals forces are electrodynamic forces which are ubiquitous between all interacting materials. They are extremely strong at short range, and retard rapidly with separation distance (Visser, 1995). The van der Waals forces are a composite of the following types of forces:

Keesom-orientation van der Waals forces

A Keesom orientated force is induced when two molecules, which both exhibit dipole moments, orientate themselves so that the positive pole of one is directed towards the negative pole of the other (permanent moment / permanent moment interactions).

Debye-induction van der Waals forces

A Debye induction force arises when a molecule which has a dipole moment, induces an electrical dipole in a neighbouring apolar molecule, provided that the latter is polarizable (permanent moment / induced moment interactions).

Dispersive van der Waals forces

Also known as van der Waals-London forces, the dispersive van der Waals force is induced by the random movement of electrons within an electron cloud, with respect to their nuclear protons. The formation of a temporary dipole and the integration of these instantaneous moments will create an

electrical field, which in turn will induce a dipole to the neighbouring neutral molecules by polarization (instantaneous moment / induced moment interactions).

The adhesive van der Waals force (F_{vdw}) between a spherical particle and a plane surface in vacuum for example, may be expressed by the following equation:

Equation 1.1

$$F_{vdw} = \frac{Ad}{6r^2}$$

where d is the diameter of a particle that is separated by a distance r from a plane surface, and A is Hamaker's constant which represents the interaction energy that is dependent on the molecular properties of both interacting materials such as the number of atoms per unit volume of particles, the frequency of the interacting electronic oscillators and the polarisability of molecules.

The van der Waals force (F_{vdw}) between two ideally smooth spherical particles exhibiting diameters d_1 and d_2, separated by a distance r in vacuum can be expressed by the following equation:

Equation 1.2

$$F_{vdw} = \frac{A}{12r^2}\left(\frac{d_1 d_2}{d_1 + d_2}\right)$$

As seen from both equations, the separation distance r of interacting surfaces is a critical parameter. With the influence of the separation distance being the square power in the denominator, suggests that the magnitude of van der Waals forces will significantly decrease as the separation distance increases. Moreover, indicating the possibility for manipulating of van der Waals forces by the presence of large asperities that would maintain a greater separation distance between the cores of the two interacting bodies.

1.2.1.2 Electrostatic forces

Electrostatic forces may result from the contact and separation of two different contiguous surfaces exhibiting different energy states. The electrostatic charge induced is termed as the process of triboelectrification, and is often used to describe the process of a surface gaining an electrical charge due to contact, sliding or friction with another surface. These charges can either arise from the contact of an uncharged particle with a charged particle, or the contact of two uncharged particles that have different work functions. The processing of pharmaceutical powders, such as high sheer mixing, sieving or micronisation will most likely induce triboelectrification of particulate surfaces (Carter *et al.*, 1992; Staniforth, 1994; Byron *et al.*, 1997). The simplest form of electrostatic force occurs when two charged particles come into contact, they will be subjected to either attraction or repulsion depending on their electrical charge signs. The magnitude of the electrostatic force (F_{el}) between them could be simply described by applying Coulomb's law:

Equation 1.3

$$F_{el} = \frac{q_1 q_2}{4\pi\varepsilon d^2}$$

where q_1 and q_2 are the electrical charges on the two particles respectively, ε is the permittivity and d is the distance of separation.

Another form of electrostatic forces occurs when a particle with either a positive or negative charge q encounters an uncharged particle of radius r, the first particle will induce what is called an image charge on the second particle. The electrostatic force (F_c) between these two particles can be described by the following equation:

Equation 1.4

$$F_c = \frac{q^2\left(1 - \frac{d}{\sqrt{r^2 + d^2}}\right)}{16\pi\varepsilon d^2}$$

where d is the distance between the charged and uncharged particles.

When the uncharged particle is a flat earthed conducting surface ($r \rightarrow \infty$), the charged particle will also induce an image charge on the plane surface, and the relationship becomes:

Equation 1.5

$$F_c = \frac{q^2}{16\pi\varepsilon d^2}$$

Finally, when two uncharged solids come together and are separated by a small distance, an electrostatic force may be induced upon contact, provided that they exhibit different work functions. Electrons from the material with the lower work function will be transferred to the material with the higher one, until eventually equilibrium is reached (Visser, 1989; Visser, 1995). In this case, the electrostatic forces of attraction (F_w) can be expressed by the following equation:

Equation 1.6

$$F_w = \pi\varepsilon r \frac{(\Delta U)^2}{d}$$

where ΔU is the potential difference arising from the difference in work functions, r is the radius of the interactive particles and d is the distance of separation.

Indeed, electrostatic forces were found to exert significant implications on the behaviour of pharmaceutical powders (Staniforth, 1994; Mackin *et al.*, 1997; Price *et al.*, 2000; Rowley, 2001). Pharmaceutical ingredients (both active

and excipients), are generally insulators. The low conductivity of these materials effectively limits surface charges from dissipating into the particle core, and thus play a critical role in particulate interactions. If present, electrostatic forces between drug and excipient particles in a powder system are of crucial importance during the initial blending of the powder formulations. Furthermore, a study carried out by Staniforth and Rees indicated that electrostatic charges created by surface interactions between particles played an imperative role in maintaining the stability of the ordered mixes. Interestingly, ordered mixes that were subjected to some degree of triboelectrification were less prone to blend segregation compared to uncharged powders. Furthermore, triboelectrified blends exhibited reduction of the error limits between powder samples, which is of practical significance when considering the dosage content uniformity of pharmaceutical powder systems (Staniforth and Rees, 1982).

1.2.1.3 Capillary forces

Moisture tends to adsorb to a wide variety of polar materials, leading to the formation of hydrogen bonds. The adsorption, however, is highly dependant on the partial water vapour pressure (relative humidity) of the surrounding atmosphere and the hydrophilic/hydrophobic nature of the surface of the material. Condensation of water vapour onto surfaces may occur over a wide range of relative humidities, however it is considerably increased at higher levels of relative humidity. If the condensation of the water vapour is significant, the formation of a liquid bridge may arise between two contacting surfaces due to capillary interaction, leading the formation of a meniscus force (Coelho and Harnby, 1978; Schubert, 1984).

For two smooth spherical particles with radius r that are wetted by a liquid with a surface tension of γ and a contact angle α as diagrammatically represented in Figure 1.2, the force due to the formation of a liquid bridge can be expressed by the following equation:

Equation 1.7

$$F = 2\pi\gamma r\cos\alpha$$

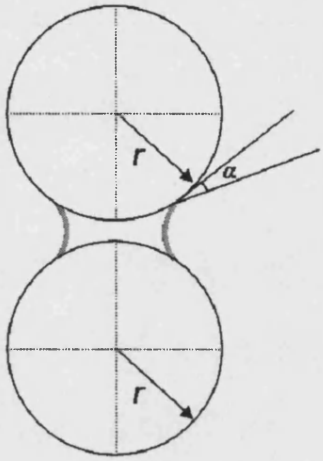

Figure 1.2. Diagrammatic representation of liquid bridging between two spherical particles.

In a similar manner, if water vapour condenses on the interface between a particle and a surface Figure 1.3, the meniscus thereby formed will correspond to the capillary force that will be added to the already present interaction forces, such capillary force is described by the following expression:

Equation 1.8

$$F = 2\pi\gamma r(\cos\alpha + \cos\beta)$$

where α and β are the contact angles of the liquid with the surface and particle respectively as shown in Figure 1.3.

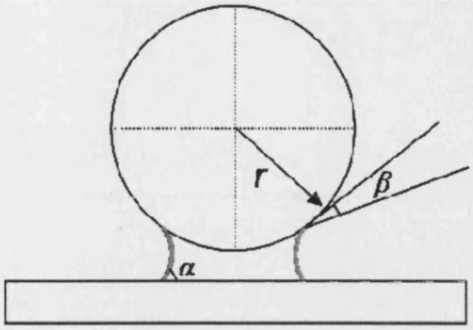

Figure 1.3. Diagrammatic representation of liquid bridging between a particle and a surface.

1.2.1.4 Mechanical interlocking

Although not a force, another type of particulate interaction which becomes more significant when the surfaces of the interacting particles are rough on a micrometer scale is mechanical interlocking. Mechanical interlocking is a mechanism which increasingly needs to be taken into consideration in regards to pharmaceutical powders, since processed powders usually exhibit a high degree of surface roughness. If such particles come into contact with one another, the surface asperities of the different interacting particles may mechanically interlock with one another. Furthermore, the contact area between these asperities will only constitute a relatively small fraction of the total surface area of the particle. Since the interactive force will be concentrated over a small surface area, the load exerted on these contact points will lead to an extremely high pressure over the contact areas. The pressure developed at these contacts may exceed the yield value of the material allowing it to flow. The flow will create a more exposed area for contact. This in turn will provide means of increasing the distribution of the load, until the applied force is insufficient to cause the particle to further yield (Cheng *et al.*, 2002; Cheng *et al.*, 2003).

Mechanical interlocking is even more critical if interacting particles possessed markedly variable dimensions. For example, if respirable sized drug particles

are adhered to coarse carrier particles, whose surface asperities are larger than the diameter of the drug particles, this may lead to the formation of stronger inter-particulate bonds due to the entrapment of the fine drug particles within the surface clefts and indentations of the coarse carrier particle. Such an interaction is considered to be more significant than the interactive forces induced by the van der Waals, electrostatic and capillary forces for non-mechanically interlocked particulate interactions (Mullins *et al.*, 1992; Podczeck, 1996; Zeng *et al.*, 2001a).

Theoretically, it is clear that particle interactions are a result of various mechanisms which contribute to particle adhesion. It is, however, rather onerous to quantify the dominant force, since many factors are involved in such interactions.

1.2.2 Factors influencing inter-particulate interactions

In light of the fact that inter-particulate interactions are indeed ruled by a number of mechanisms, several studies have outlined some of the major factors which may influence the cohesion/adhesion characteristics of respirable sized particles (Staniforth *et al.*, 1982). These mainly relate to the physicochemical properties of interactive particles, such as particle size, particle shape, surface texture, electrostatic propensity and moisture sorption properties (Ganderton and Kassem, 1992; Podczeck, 1997; Podczeck, 1999; Chew *et al.*, 2000; Zeng *et al.*, 2001a; Chew and Chan, 2002). Inter-particulate forces within the powder formulation have also been shown to be affected by the morphological properties of the drug and carrier particles (Kawashima *et al.*, 1998b; Kawashima *et al.*, 1998c; Louey *et al.*, 2003), presence of ternary components (Tee *et al.*, 2001; Louey *et al.*, 2002), drug-carrier ratio (Steckel and Müller, 1997b), blending process (Staniforth, 1987; Zeng *et al.*, 1996a) and environmental conditions during processing and storage (Young and Price, 2004). Further information regarding some of the main specific influences on particulate interactions are highlighted below.

1.2.2.1 Particle size

It is apparent that the larger the particle the stronger the influence it will have on adjacent objects. For example, van der Waals forces are directly proportional to the radius of the particle (Lam and Newton, 1992). The specific influence of particle size on interaction forces has been investigated by Podczeck *et al.* (Podczeck *et al.*, 1994; Podczeck *et al.*, 1995). The performance of a powder is usually determined by the relative influence of inter-particulate forces in comparison to the gravitational forces acting upon the mass of the particle. The powder behaviour will, therefore, be directly affected with particle size, particularly when the dimensions of a particle are reduced to such an extent where particulate interactions become more significant than the gravitational influence which acts as separation forces on particles. Although dependant on physicochemical properties of a specific material, this transition from the macroscopic influences to the microscopic generally occurs for particles smaller than 60 μm (Kassem *et al.*, 1989; Podczeck, 1998b; Zeng *et al.*, 1999). Thus, the cohesive and adhesive properties of particles with a diameter less than 10 μm are governed by the van der Waals forces (dominated by the London dispersive forces) and the relative influence of the dynamic capillary and electrostatic forces. These physical forces govern why fine powders are intrinsically cohesive and, thus, exhibit poor flow properties (Ganderton and Kassem, 1992) and their propensity to adhere to solid surfaces (Lord and Staniforth, 1996; Staniforth, 1996b).

1.2.2.2 Particle shape

In powder technology, it is almost always the case that the methods utilised in the production of particles give rise to particles with various geometrical properties. Thus, the resulting particle shape is considered as one of the most unreliable and uncontrollable factors which may influence inter-particulate interactions. An increase in the separation distance between

particles will directly result in a decrease in inter-particulate forces (Podczeck, 1998a). Whether particles exhibit a regular shape or not, the contact area between interacting surfaces and the tendency of particles to orientate themselves to the most stable conformation upon the application of an external energy play a significant role in controlling the adhesive properties of powders (Podczeck, 1996; Staniforth, 1996b).

1.2.2.3 Surface energy

The surface energy of a solid is defined as the free energy change during the increase of the surface area by one unit *in vacuo*. The excess energy is due to force imbalances rising at the surface of the material. Although a solid is rigid and resistant to stress, in a similar manner to the surface tension of a liquid, it possesses a surface energy resulting from the net imbalance of surface forces. The difference between a liquid surface and a solid surface, is that the molecules on the surface of a liquid move more freely than those on the surface of a solid, therefore, a consistent surface energy is omnipresent over the entire surface of the liquid, which is numerically equal to the surface tension of the liquid. Due to the considerable rigidity and reduced mobility of surface molecules of a solid, surface energy is unequally distributed over the solid surface. Regions such as edges and surface asperities may possess a higher energy than regions of plane surfaces. The level of variation in surface energy of a solid is highly dependent on the processing and treatment procedures which the material was subjected to such as crystallisation, milling, dissolution or condensation (Buckton *et al.*, 1988; Ohta and Buckton, 2004).

In general, solids exhibiting high surface energy are more likely to form strong inter-surface forces. Both cohesion and adhesion of solids are strongly related to the surface energies of interacting objects. The minimum work required for the separation of two surfaces and therefore, the energy bonding them together is equal to the difference in free energy before and after

separation. Such difference or change in free energy is defined as the work of adhesion W_a. The work of adhesion, W_a, between two materials C and D is given by:

Equation 1.9

$$W_a = A\left(\gamma^C + \gamma^D - \gamma^{CD}\right)$$

where A is the area produced by separation, γ^C and γ^D are the free energies per unit surface area of solids C and D in air respectively, and γ^{CD} is the free energy of the C-D interface per unit area. Thus for the same material C, the work of cohesion, W_c is described by the following equation:

Equation 1.10

$$W_c = 2A\gamma^C$$

Surface energy appears to exert a critical influence on the cohesive/adhesive properties of drug-drug and drug-excipient systems, considerably affecting mixing homogeneity as well as dispersion, de-aggregation and aerosolisation behaviour (Ahfat *et al.*, 1997; Cline and Dalby, 2002).

1.2.2.4 Relative humidity

As previously discussed, the tendency for moisture to adsorb onto the surface of pharmaceutical dry powder particulates is significantly dependent on the relative humidity (partial water vapour pressure) of the surrounding environment, and the hydrophilic/hydrophobic characteristics of the surface of the material. Recent studies have focused on the role of relative humidity on the adhesive properties of powders (Hindle and Makinen, 1996; Price *et al.*, 2002a; Price *et al.*, 2002b; Harjunen *et al.*, 2003; Iida *et al.*, 2004a; Young and Price, 2004). Relative humidity (RH) influences inter-particulate forces through two distinct mechanisms (Price *et al.*, 2000). If a significant amount of water condenses between the surfaces of interacting particles, capillary

forces may dominate particulate adhesion. However, the presence of a layer of water on the surface of interacting particles would increase the conduction of insulating materials and, thus, possibly dissipate the electrostatic charges which may be present upon contact. A relative humidity in the range of 40 to 50% is considered to be the optimum environmental conditions for maintaining a lower degree of capillary and electrostatic influences. Above 65% RH, capillary forces may significantly increase particle adhesion. At low humidities (< 30% RH) particle adhesion may be more susceptible to electrostatic forces due to triboelectriifcation upon powder handling (Podczeck *et al.*, 1997a; Podczeck *et al.*, 1997b). A more detailed review of the influence of relative humidity on inter-particulate forces therefore performance of dry powder formulations is presented in Chapter 5.

1.2.2.5 Surface texture and contact area

Since particulate interactions are effectively a surface phenomenon, the morphology of the surface will have a critical effect on the adhesive properties of interactive particles (Kawashima *et al.*, 1998a; Zeng *et al.*, 2000a; Price *et al.*, 2002b; Iida *et al.*, 2003b; Larhrib *et al.*, 2003a; Iida *et al.*, 2004b). Theoretical predictions of particulate interactions are usually based on the assumption that particles exhibit an ideal spherical shape with a smooth surface, or that a spherical particle adheres to a perfectly flat smooth surface (Wen and Kasper, 1989; Wang, 1989; Chew and Chan, 2001). However, in reality this is not the case, since pharmaceutical solids are known to exhibit a diverse variety of shapes and surface topographies. Particle-particle and particle-surface interactions can be classified into three main cases: (*a*). Spherical particles adhering to either a smooth surface or coarser spherical particles having a smooth surface, only in such a case it is possible to quantify adhesive forces acting between interacting surfaces. (*b*). Spherical or irregularly shaped particles adhering to a surface with relatively small scale asperities, which would dramatically decrease the contact area, thereby, increasing the separation distance and consequently decreasing

adhesive forces. (*c*). Spherical or irregularly shaped particles adhering to a surface with relatively large scale asperities. In this case, adherent particles may mechanically interlock, leading to an increase in contact area and a decrease in separation distance between the two interacting bodies, resulting in higher adhesive forces (Otsuka *et al.*, 1988; Wen *et al.*, 1989; Taheri and Bragg, 1992; Mizez, 1994).

In view of the above, if we examine a carrier-based dry powder inhaler system for example, some of the fine drug particulates may adhere to smooth regions of the carrier surface whilst others may adhere to rougher sites displaying relatively small scale asperities, or even fall therefore become entrapped into bigger scale crevices that can accommodate them (Figure1.4). When exposed to external forces such as turbulent air fluidisation, particles adhering to smaller scale asperities may be easier to dislodge disperse and de-aggregate from the surface of carrier particles compared to those attached to the smooth regions, again due to increased contact area in the later situation thus significant increase in particle-particle adhesion. In contrast however, particles entrapped in the bigger scale crevices are the most difficult to remove, therefore requiring the highest energy to dislodge them from the surface of the carrier particle. In light of the above, it was found that it is rather imperative to obtain an optimised degree of surface rugosity in order to attain a desirable balance between adherence and detachment of drug particulates from the surface of carrier particles (Kassem and Ganderton, 1990; Byron *et al.*, 1996; Staniforth, 1996; Heng *et al.*, 2000; Zeng *et al.*, 2001c; Clarke *et al.*, 2002).

A more comprehensive understanding of the role of surface morphology is necessary to controllably modify the efficiency of drug delivery from carrier based dry powder inhalation formulations.

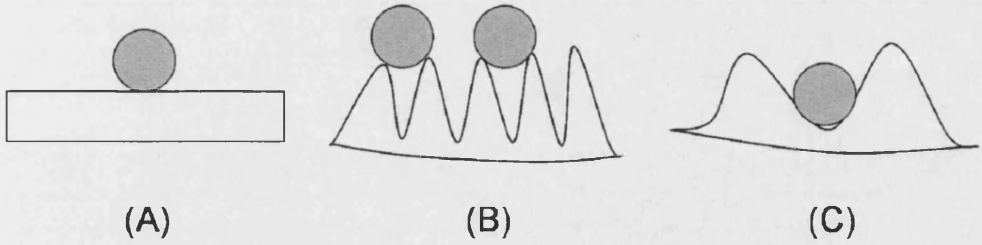

Figure 1.4. Diagrammatic representation of different particle-surface interactions. Spherical particle adhered to a flat smooth surface (A), spherical particles adhered to a surface with relatively small scale asperities (B) and finally entrapment of a particle within a large scale crevice (C).

1.3 DPI formulation development

The basic concept of applying local medical treatment to the respiratory tract *via* the inhalation of 'dusts' evolved as a consequence of the therapeutic use of penicillin in the late 1940s (Bell, 1992). Despite numerous attempts to develop devices that would effectively deliver such therapeutic clouds, it wasn't until the 1960s, that the first successful dry powder inhaler, the Spinhaler® was introduced for the delivery of high dose disodium cromoglycate (Bell *et al.*, 1971).

Over recent years, dry powder inhalers (DPIs) have successfully undergone further advances, since they were genuinely considered as favourable systems for pulmonary delivery over existing conventional pMDIs and nebulisers. For example, dry powder inhaler systems overcome the use of environmentally harmful volatile propellants, such as CFCs or HFAs. Furthermore, DPIs are considered to be more patient friendly, since dispersion and entrainment of drug particles entirely depends on the inhalation efforts of the patient, therefore alleviating problematic issues of actuation and inhalation synchronisation. Moreover, DPIs are relatively inexpensive and not cumbersome as nebulisers, thus highly portable. In

addition the engineering design of DPI devices allows the possible delivery of high drug doses (Timsina *et al.*, 1994; Ashurst *et al.*, 2000).
As previously discussed, and given the specific particle size of drug particulates (≤ 5 μm) that is vital for the deep delivery to the conducting airways, respirable-sized particles are intrinsically cohesive due to their large surface area and the dominance of the physical forces acting. Such pronounced cohesiveness renders the powder poorly flowable and hinders the ease of handling during manufacturing processes such as mixing and capsule filling (Ganderton and Kassem, 1992). The most common and reasonable solution that would address this shortcoming, is to co-process the fine drug particulates with larger carrier particles of an inert excipient. The carrier particles are usually designed to be of such a size that they deposit in the oropharynx after inhalation (Hinds, 1999).

In order for the drug particulates in a carrier-based DPI system to reach the desired regions of the respiratory tract, they must detach from the surface of the carrier particle during inhalation, otherwise they are most likely to face the same fate as the carrier particles, that is impaction in the upper airways. This key process is critically dependent upon the adhesive bonding that is created between the drug and carrier particulates. This emphasises the importance of attaining some sort of balance between the inter-particulate forces encountered in an interactive binary mixture (Staniforth *et al.*, 1982; Begat *et al.*, 2004; Dickhoff *et al.*, 2005). The drug particulates need to be sufficiently adhered to carrier particles during mixing, in order to aid in blend homogeneity, accurate dose metering, device filling, also preventing segregation and formulation instability upon handling and storage. Yet, at the same time, drug particulates need to be able to be detached from the carrier particles upon actuation to form a fine particle cloud. It is this balance of particulate interaction that is required for efficient and successful development of DPI systems (Ganderton and Kassem, 1992; Prime *et al.*, 1997; Clarke *et al.*, 2002).

1.3.1 Carrier particles

Carrier particles are mainly utilised as a bulking agent (a 'diluent') to improve the flowability of the active ingredient, aid in accurate dose metering and enhance efficient device clearance. Although a number of excipient materials have been used as carriers (diluents), the traditionally used lactose remains the most commonly and frequently used carrier in pharmaceutical preparations of dry powder inhaler formulaitons. This is mainly because of its history as an inexpensive, inert, stable and safe excipient (Timsina *et al.*, 1994; Karhu *et al.*, 2000).

For currently marketed dry powder formulations, the only approved excipients are lactose and glucose (British Pharmacopoeia, 1993). However more recently, several studies have been conducted as to the evaluation of other types of sugars in DPI systems such as mannitol, sorbitol and trehalose (Naini *et al.*, 1998; Chew and Chan, 1999; Tee *et al.*, 2000; Bosquillon *et al.*, 2001; Steckel and Bolzen, 2004).

The materials used as carriers for DPI formulations should exhibit a high degree of chemical and physical stability, be readily available in an acceptable pharmaceutical grade and with low hygroscopic characteristics. In addition, the excipient materials should easily metabolised and cleared from the airways upon inhalation delivery. In most cases, lactose particles are prepared by crystallisation, and can therefore be obtained in a variety of shapes and sizes (Jelen and Coulter, 1973a; Jelen and Coulter, 1973b; Raghavan *et al.*, 2000; Raghavan *et al.*, 2001). Lactose is known to have two anomeric forms, namely α and β, both are present in aqueous solution with α/β ratio close to 40/60, but only the α-lactose crystallises spontaneously below 93.5°C. α-Lactose monohydrate is considered to be a preferential carrier in DPI formulations due to its particular "tomahawk" shape of its crystals which allows significant area for drug-excipient interaction. Typical "tomahawk" shaped crystals of α-lactose monohydrate are shown in Figure 1.5.

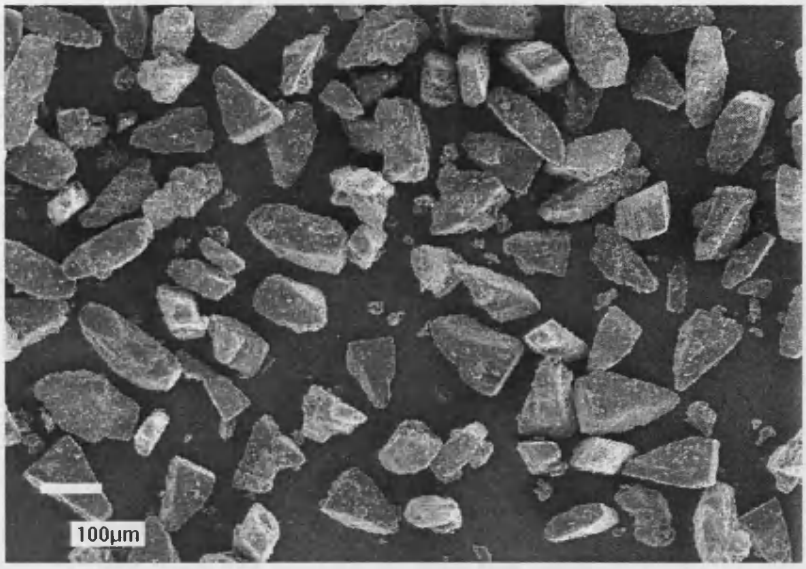

Figure 1.5. Representative scanning electron photomicrograph of commercial crystalline grade α-lactose monohydrate (Lactochem®) particles.

1.3.2 Carrier particle size

On account of the fact that particle size is one the most crucial elements affecting inter-particulate interactions, the size of lactose carrier particles has been found to affect the delivery of drug particulates within a DPI system. Lactose can be readily produced in a wide range of particle size distributions. However, inhalation grade lactose is most commonly produced in the particle size range between 63 and 90 µm (Bell *et al.*, 1971). Several studies have focussed on the influence of the size of carrier particles on the delivery performance of DPI formulations (Kulvanich and Stewart, 1987; Zeng *et al.*, 2000b; Clarke *et al.*, 2001; Dickhoff *et al.*, 2003; Islam *et al.*, 2004b; Louey *et al.*, 2004a; Louey *et al.*, 2004b).

Kassem *et al.* for example, investigated the effect of different size fractions of lactose on the respirable fraction of salbutamol sulphate from 1:67.5 ordered mixtures tested at various flow rates. The investigation indicated that the smaller sized carrier particles showed a higher respirable fraction of

salbutamol sulphate regardless of the variation in flow rate (Kassem *et al.*, 1989). Such results were in accordance with those obtained by Steckel and Müller. Their study also indicated that reduction in carrier size was reported to improve budesonide aerosolisation (Steckel and Müller, 1997b). In a similar manner, increased deposition profile of disodium cromoglycate was observed following the use of smaller size ranges of lactose and glucose carriers (Braun *et al.*, 1996). In addition, higher fine particle fraction of salbutamol sulphate was obtained from carrier particles exhibiting a size of less than 63 µm, compared to those in the size range of 63-90 µm (Zeng *et al.*, 2000b).

Although not well understood, a plausible explanation why drug particulates are easily detached from finer carrier particles may possibly be that adhesive inter-particulate forces are directly influenced by the decrease in particle size. Another possible suggestion is that drug particulates adhering to larger carrier particles are more liable to loss due to impaction of the later onto inhaler walls and upper conducting airways. Furthermore, it is thought that fine carrier particles can more easily disrupt the agglomerates of drugs exhibiting high cohesive nature (Begat *et al.*, 2004).

The influence of carrier particle size is rather controversial, since findings of some other studies were contradictory to the aforementioned literature. For example, a study conducted by Byron *et al.* indicated that coarser lactose particles (53-105 µm) were shown to yield a higher deposition profile of terbutaline sulphate compared to finer lactose particles (≤ 53 µm) (Byron *et al.*, 1990). It is significantly important to note however, that the concentration of fine lactose particulates should be controlled, since an increase in the deposition profile of the drug upon increasing the carrier particle size may be in part due to the presence of considerable amounts of fine carrier particulates. It is extremely crucial, therefore, that such studies are conducted on the effect of particle size of inherent carriers under equivalent conditions, that is to say similar concentrations of fine carrier particulates.

1.3.3 Addition of fine excipient particles

Over recent years, extensive and meticulous investigations have been conducted as to the exploration of the influence of fine carrier particulates on the performance efficiency of DPI formulations. In fact, the inclusion of controlled and specified amounts of fine particulates is considered as one of the most significant and well recognised advances in the optimisation of pulmonary drug delivery. Scientific literature is enriched with various studies aimed at the methodical scrutiny of its critical influence in DPI systems (Clarke *et al.*, 1998; Lucas *et al.*, 1998b; Zeng *et al.*, 1998; Zeng *et al.*, 1999; Tee *et al.*, 2000; Zeng *et al.*, 2000c; Louey and Stewart, 2002; Islam *et al.*, 2004a).

In order to obtain a more fundamental insight into the effect of added fine carrier particulates, some studies have involved a pre-treatment step to remove intrinsic fines from carrier particles. Such pre-treatment procedures have involved either air jet sieving, direct blowing of compressed air through coarse carrier particles. Certain doubts however have been raised as to the efficiency of such procedures in removing the presence of intrinsic fine particulate material.

A study carried out by Larhrib and co-workers investigated the aerosolisation efficiency of salbutamol sulphate from formulations exhibiting different grades of coarse lactose. The formulation displaying the highest proportion of fine particulates (< 5 μm) was found to yield the greatest respirable fraction of salbutamol sulphate upon inhalation (Larhrib *et al.*, 1999). Similar findings were also observed in a study by Tee and co-workers which investigated the influence of fines not only in lactose based formulations, but also with mannitol and sorbitol coarse carriers. Again formulations containing coarse carriers with the highest proportion of fine particulates resulted in improved drug aerosolisation efficiency (Tee *et al.*, 2000).

Another critical issue that has been taken into consideration is the concentration (percent w/w) by which fines are included to powder

formulations. The inclusion of 1.5 to 9% w/w of fine particulates generally resulted in a significant increase in fine particle dose of the drug (Zeng *et al.*, 1998). Similarly, the observation of a study carried out by Islam *et al.* suggested that the highest fine particle fraction of salmeterol xinafoate was achieved upon the inclusion of 15% w/w fine lactose particles. Coarse lactose particles in this study were processed by a wet decantation with alcohol procedure to remove intrinsic fine particulates (Islam *et al.*, 2004a; Islam *et al.*, 2004b).

Such findings clearly highlight the significance of optimising the amount of fines incorporated in a DPI formulation. There are, however, several related issues to the addition of fines. Studies have indicated that their addition may affect the flow properties of the formulation (Lucas *et al.*, 1998a; Tee *et al.*, 2000). Furthermore, increasing concentration of fines was found to increase the fraction of drug retained in the inhaler device. Such observations have been attributed to the increased adhesive inter-particulate interactions between fine carrier and drug particulates (Podczeck, 1999).

A number of theories have been employed in order to try to gain a better understanding of the ambiguous mechanisms by which fine carrier particulates tend to enhance the aerosolisation efficiency of carrier based systems. One of the proposed theories relies on the concept of the 'active sites' phenomena (Srichana *et al.*, 1998b; Zeng *et al.*, 1998; Bennett *et al.*, 1999; Larhrib *et al.*, 1999; Tee *et al.*, 2000; Zeng *et al.*, 2001c). This theory suggests that fine carrier particulates preferentially adhere to high surface energy strong adhesion 'active sites', thus forcing drug particulates to bind to the low energy weak adhesion sites. The 'active sites' theory is presented and discussed in more detail in Chapter 4. Upon patient inspiration, the weakly bound drug particles are more easily liberated from the surface of the carrier particle, thereby facilitating more efficient aerosolisation.

Zeng *et al.* highlighted that formulations produced by first blending the coarse and fine carrier particles resulted in higher fine particle fractions than those produced by first blending the coarse carrier and drug particles (Zeng *et al.*,

1999; Zeng *et al.*, 2000c). Meanwhile, Lucas *et al.* revealed that for certain active ingredients, the order of blending is of no true significance and had no apparent effect on the performance of ternary DPI formulations. In fact, it was suggested that fine carrier particles and drug particles tend to redistribute themselves over the coarse carrier surface to form aggregated colonies or what is called 'fine particle multiplets' (Lucas *et al.*, 1998a). The premise of 'fine particle multiplets' was also supported by Soebagyo and Stewart and further advocated by of the studies of Louey and Stewart. Such 'multiplets' are considered to exhibit the desired effect of being more easily detached from coarse carrier particles since they exhibit a greater detachment mass, thus higher drag forces and kinetic energies, therefore tend to overcome adhesion with the coarse carrier and de-agglomerate more easily (Soebagyo and Stewart, 1985; Louey and Stewart, 2002).

1.3.4 Effect of surface roughness on drug delivery

The surface roughness of carrier particles is undoubtedly one of the most crucial determinants governing particulate interactions *via* either cohesion or adhesion. The bulk manufacturing of most commercially available excipients involve the subjection of excipient materials to a number of unavoidable rather vigorous industrial procedures such as mechanical milling. As a result, physical instability is commonly introduced. Such physical instability could manifest itself in various aspects. One is that particles tend to exhibit evident changes in the topographical features such as apparent surface irregularities due to the presence of crevices (fissures, peaks and troughs). Another aspect of physical instability lies in the introduction of crystalline disorder, thus occurrence of variations in surface free energy (Begat *et al.*, 2003). Such thermodynamically unstable regions on the surface are the likely to engage drug particulates during mixing *via* the influence of their high surface free energy. As previously introduced, if carrier particles tend to display a rough surface structure on a micrometer scale, then the fraction of drug particulates entrapped into the carrier surface crevices is almost unlikely to

become dislodged under the application of turbulent airflow due to strong adherence *via* mechanical interlocking. Furthermore, it is thought that such crevices also act as a shelter for any entrapped drug particulates rendering them unavailable to the drag forces generated by inhalation air stream. In view of the above and given the unpredictable effect of physical instability due to variations in surface rugosity, it may be beneficial to carry out some sort of pre-treatment of carrier materials, prior to their use in DPI formulations. Such treatments are thought to possibly limit *inter* and *intra* batch variations of carrier materials, thereby, enhancing uniformity and reproducibility of *in vitro* and *in vivo* performance of DPI formulations.

Kassem and Ganderton studied the aerosolisation performance of salbutamol sulphate from formulations exhibiting three kinds of lactose carriers having different surface smoothness. The highest level of surface smoothness was demonstrated by lactose particles that were recrystallised from solution. It was further reported that the highest respirable fraction of drug particulates (< 5 μm) was obtained from formulations employing lactose carriers with a lower degree of surface rugosity (Kassem and Ganderton, 1990).

Indeed, physical characterisation of α-lactose monohydrate carrier particles, in terms of their surface roughness, has been shown to be of significant importance with regards to manipulating the adhesive properties governing particulate interactions in a powder formulation (Kawashima *et al.*, 1998a; Podczeck, 1998c; Zeng *et al.*, 2000a; Ferrari *et al.*, 2004; Flament *et al.*, 2004). However, the degree of surface roughness of the carrier particles should ideally be optimized to manipulate the inter-particulate surface adhesion to allow high therapeutic delivery of the active ingredient, while exhibiting sufficient adhesion in preventing segregation of the powder upon handling (Ganderton and Kassem, 1992).

Over recent years, various attempts have been carried out in order to modify the surface characteristics of lactose carrier particles. Previous attempts include increasing surface smoothness *via* complete recrystallisation of

carrier particles (Kassem and Ganderton 1990), high speed wet granulation (Colombo *et al.*, 2000; Ferrari *et al.*, 2004), crystal engineering involving modifying both the shape and surface rugosity of lactose particles through crystallization (Larhrib *et al.*, 2003a) and surface treatment achieved by wetting with an aqueous-alcohol solution (Iida *et al.*, 2003; Islam *et al.*, 2004a). Furthermore, surface treatment *via* the use of ternary components such as magnesium stearate and leucine was also investigated (Staniforth, 1996; Lucas *et al.*, 1999; Young *et al.*, 2002). One problem with the resulting particles, however, is that they may possibly exhibit relatively unpredictable alterations of surface morphologies due to relative difficulties in controlling the processing and treatment conditions. Chapter 3 focuses on the various methodologies utilised in modifying carrier surface morphological features and its implications and their influence on carrier-drug inter-particulate interactions and, thus, formulation performance.

1.4 Aim of the study

In spite of the successful advances in the area of inhalation technology over many years, one of the primary goals remains. That is the development of an expedient, efficient dry powder inhaler system with a reproducible performance, independent of the active ingredient. Formulations aspects such as optimising physicochemical characteristics of drug and carrier particles and achieving *inter* and *intra* batch equivalence were found to substantially minimise undesirable repercussions on drug delivery performance.

The surface rugosity of an excipient particle, in a carrier based inhalation formulation, has been shown to play a critical role in the behaviour of respirable drug particles. The surface texture of a commercial grade carrier particle is usually rough on a micrometer scale, with an array of asperities and clefts on its surface. These sites are thought to be related to areas of high surface free energy, providing a desirable and preferential area to which

active drug particles are readily attracted and strongly adhered. It would, therefore, be highly advantageous to dramatically decrease the degree of high surface free energy sites by producing a surface with a well-defined surface roughness on a nanometre scale.

The primary aim of the study is to investigate the influence of nanometre scale roughness on drug-excipient adhesion properties and consequently the aerosolisation properties of respirable particles. A novel temperature controlled surface etching process has been developed to controllably modify the surface smoothness of commercial grade excipient particles. Qualitative characterisation of the surface morphology of the modified excipient particles was determined through a number of methodologies. Quantitative analysis of the deposition profile of model drug particles from different surface-smoothed excipient particles was also performed.

Moreover, the potential use of surface etched lactose carriers for low dose drug delivery was further studied. Additional investigative work was also dedicated to achieve a more comprehensive understanding of the prospective influence of environmental conditions on the stability and reproducibility of DPI formulations, and the manner in which the physicochemical properties of the excipient materials play in the overall stability of a DPI formulation.

Chapter 2

General materials and methods

2.1 Materials

2.1.1 Pharmaceutical excipients

α-Lactose monohydrate (Lactochem®) was supplied by Borculo Whey (Chester, UK). The lactose was vibrated through a nest of sieves to obtain 63-90 μm sieve fraction, which was used throughout the study. Fine particle α-lactose monohydrate (Sorbolac 400) was supplied by Meggle (Wasserburg, Germany). α-Lactose monohydrate is a disaccharide and its chemical structure is shown in Figure 2.1.

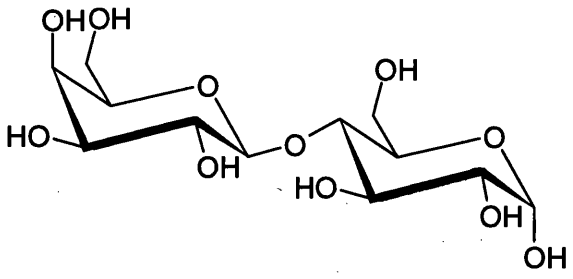

Figure 2.1. Chemical structure of α-lactose monohydrate.

2.1.2 Active pharmaceutical ingredient

Salbutamol sulphate was the therapeutic active ingredient that was employed as a model drug for aerosolisation performance investigations. Micronised salbutamol sulphate was supplied by Aventis Pharma (Cheshire, UK). Salbutamol sulphate is a β_2-adrenergic agonist which acts as a bronchodilator, its chemical structure is shown in Figure 2.2.

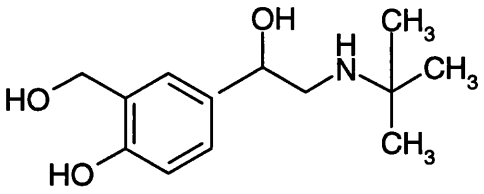

Figure 2.2. Chemical structure of salbutamol.

2.1.3 Solvents

Methanol (HPLC grade) was supplied by Fisher Chemicals (Loughborough, UK). Glacial acetic acid was supplied by BDH (Poole, UK). Ultra pure water was produced by reverse osmosis (MilliQ, Millipore, Molsheim, France). All solvents used throughout the study were at least of analytical grade.

2.2 Methods

2.2.1 Particle size analysis

A number of methods could be applied for the measurement of the particle size distribution of pharmaceutical excipients and active drug materials. Such methods include sieve analysis, time of flight determination, sedimentation,

mercury porosimetry, low angle laser light scattering (LALLS) and impaction based techniques. The methods of choice that were found purposeful for the particle size distribution measurements throughout the study were low angle laser light scattering (LALLS) and impaction based techniques, utilising two standard method; the twin stage impinger (TSI) and the multi stage liquid impinger (MSLI). It is important to note however, that LALLS determines the actual geometrical particle size, whereas impaction based techniques describe the aerodynamic diameter of particles (Hinds, 1999). The aerodynamic diameter for a particular particle is defined as the diameter of a spherical particle with a density of 1 g/cm^3 that has basically the same settling velocity as the particle in question. It is clear from the definition that the aerodynamic diameter is a standardised description for the shape (sphere) and the density (1 g/cm^3) due to the diverse irregularities of shape which most pharmaceutical ingredients exhibit. Impaction based techniques are routinely used for the *in vitro* efficiency testing of inhaler formulations performance due to the correlation between aerodynamic diameter properties and sedimentation behaviour of the aerosol particles in the lung and aerodynamic diameter (de Boer *et al.*, 2002; de Boer *et al.*, 2004; Weda *et al.*, 2004). The impinger methods are described in more detail in section 2.2.7.3.

The LALLS technique is considered as a preferable technique for the characterisation of particle size distributions in pharmaceutical research and industry (Clark, 1995b; de Boer *et al.*, 2002). In simple terms, particle size distribution analysis by LALLS involves measuring the intensity and pattern of the diffraction of a monochromatic, collimated light which is scattered following the passage of a laser beam through a particulate sample. The intensity and pattern of diffraction of the scattered light are a specific representation of an individual particle size. However, for micron-sized particles, the optical properties of the material are to be considered and light scattering in this case is described by the Mie theory (Allen, 1990). The Mie theory states that the induction of light scattering is triggered by the difference between the refractive indices of the particle and the surrounding medium.

On the whole, when a sample with a range of particle sizes is presented for analysis, the scattering pattern is obtained by integration over the whole range of single scatters caused by each particle present in the sample. Particle size distributions are then calculated by matching the experimental and theoretical diffractograms (British Standard, 1999).

All particle size analyses were determined using a Malvern Mastersizer X (Malvern Instruments Ltd, Malvern, UK). The instrument is equipped with a magnetically stirred sample cell with a capacity of approximately 20ml. A 0.1% w/w lecithin / cyclohexane solution was used as a sample dispersant. To deagglomerate cohesive powders, some samples analysed were sonicated for 5 minutes prior to measurement. A 300 mm lens was used, as it allowed the detection of particle size in the range of 1.2 to 600 µm. Approximately 1 mg of the material was suspended in the dispersant and continuously stirred in order to obtain an obscuration level of 20-30%. The particle size distribution was calculated and represented as a volume distribution, and was also characterized by the 10^{th}, 50^{th} and 90^{th} percentile of the cumulative particle undersize frequency distribution. All samples were prepared and analysed in triplicate.

2.2.2 Scanning electron microscopy

The overall morphology of α-lactose monohydrate carrier particles that were employed throughout the study was investigated using the scanning electron microscope. Scanning electron microscopy (SEM) was effectively utilised for the description of both particle shape and surface topography of the carrier particles. Scanning electron microscopy (SEM) involves the creation of a high resolution image by the use of a collimated beam of electrons instead of light waves. A beam of focussed electrons is emitted from an electron gun. Such electron beam is scanned back and forth over a conductive sample. As a result of the high speed continuous sweeping of the electrons across the specimen, secondary electrons (weakly bound conduction-band electrons)

are emitted from the surface due to the local irradiation. The signal produced by the emitted secondary electrons from the specimen is collected on a detector and interpreted to form the final image.

Representative powder samples were sprinkled on adhesive black carbon tabs, which were pre-mounted on aluminium stubs. A thin film of gold was vaporised onto the sample surface using a sputter coater (Model S150B, Edwards High Vacuum, Sussex, UK). Samples were then examined using a JEOL 6310 SEM (Japanese Electron Optics Ltd, Tokyo, Japan) at 10 KeV.

2.2.3 Specific surface area

Gas adsorption is commonly used to measure the surface area of materials by condensing the gas onto the surface and determining the amount adsorbed in producing a monolayer. The specific surface area of α-lactose monohydrate carriers were determined by nitrogen gas adsorption. For a gas of known molecular cross section area, the quantity adsorbed can easily be converted into surface area. Measuring the amount of gas adsorbed over a range of relative pressures enables the monolayer coverage to be determined by applying the Brunauer, Emmett and Teller (BET) equation (Brunauer *et al.*, 1938). The linear form of the BET equation is given by:

Equation 2.1

$$\frac{P}{V_a(P_o - P)} = \frac{1}{V_m C} + \frac{(C-1)}{V_m C}\frac{P}{P_o}$$

where P is the relative partial pressure of probe gas, V_a is total mass of gas adsorbed at the specific pressure P, V_m is the mass of adsorbed gas forming a monolayer coverage, P_o is the saturation pressure and C is the BET constant which represents the affinity of the gas to the surface of the material. Subsequently, by plotting the transform of the equation the monolayer coverage can be calculated from the slope and its intercept.

Equation 2.2

$$\frac{P}{V_a(P_o - P)} vs \frac{P}{P_o}$$

Using the BET equation to measure the surface coverage allows the calculation of the specific surface area (*sa*) by the use of the following equation:

Equation 2.3

$$sa = V_m N_A \sigma$$

where N_A is Avogadro's constant and σ is the cross sectional area of the adsorbing species, which is equivalent to 0.162 nm^2 for N_2 (Gemini 2360 Surface Area Analyzer Operator's Manual V5.0, Micromeritics). Cooling the sample to the temperature of boiling liquid nitrogen (77 K) reduces thermal effects, enables efficient condensation of nitrogen onto the surface and gives thermal stability; thereby limiting variations in the saturation vapour pressure. Prior to analysis, it is necessary to dry the sample in order to remove condensed water from all the pores. Remaining water would freeze during analysis at 77 K, blocking the access of probe gas to the pores leading to erroneous measurements.

Surface area was determined by 5-point BET N_2 adsorption using the Gemini 2360 nitrogen adsorption apparatus (Micromeritics, Norcross, USA). Samples were accurately weighed into standard glass bulbs and were then dried at 40°C to constant mass (16-20 hours) under a continuous stream of dry nitrogen using the FlowPrep 060 (Micromeritics, Norcross, USA). The partial pressure of N_2 was adjusted by mixing the analysis gas with helium, to a maximum partial pressure of 0.2.

2.2.4 X-ray powder diffraction

X-ray powder diffraction (XRPD) is the most commonly used method in investigating the order of crystal structures of pharmaceutical powder samples (Nakai *et al.*, 1977; Rowe *et al.*, 1994). XRPD briefly involves the measurement of a diffracted monochromatic X-rays as they pass through the atomic spacing which exits between the atoms in a crystalline material. The wavelength of X-rays, from 0.1 to 100 Å, covers the range of molecular and ionic bond length. The shorter wavelength of the X-ray spectrum (1 to 2 Å) is of most significance in examining crystal structures. The initial parameter measured is the angle of diffraction, which relates to the dimension of the interatomic spacing responsible for the diffraction *via* the Bragg equation (equation 2.4):

Equation 2.4

$$\lambda = 2d \sin \theta$$

where λ is the wavelength of the incident radiation, d is the interatomic spacing and θ is the diffraction angle. By determining the interatomic spacing for a known crystal structure, the three-dimensions of the unit cell can be determined (Guinier, 1994). Due to the fact that XRPD reflects the given crystal structure, it can be used to determine the presence of different crystal structures and/or crystalline disorder such as the presence of amorphous regions. However, the use of X-ray diffraction spectra in the direct quantification and detection of the amount of amorphous materials present has been proved to be relatively unsuccessful (Saleki-Gerhardt *et al.*, 1994). Although the strength of the diffraction increases as the volume of crystalline regions increases, the signal strength is subjected to spherical aberration as a result of the geometry of the apparatus.

X-ray powder diffraction spectra of α-lactose monohydrate samples were collected using a Phillips X-ray powder diffraction system (D5000, Bruker AXS, Cheshire, UK). The system consisted of the following components; 4 kW X-ray generator (PW 1730/00), long fine-focus Cu target (PW 2273/20,

λ=1.504 Å), computer-controlled vertical diffraction goniometer (PW 1820/00) and Xe proportional counter (PW 1711/10) with a graphite monochromator (PW 1752/00). Settings were as follows; 2° to 39.960° 2θ, step size 0.036° 2θ, step time 0.5 seconds, temperature 25°C.

2.2.5 Powder bulk and tapped density and flow

The flowability of pharmaceutical powders is considered as one of the most crucial characteristics, which influences more or less all industrial handling procedures that are fundamentally encountered with formulation development. In dry powder inhaler formulations, flow properties of both drug and carrier particles play a critical role in attaining effective drug content uniformity, since powders that flow well tend to produce mixtures with a better degree of order. Furthermore, good flow properties ensure reproducible capsule filling and possibly improved aerosolisation efficiency. The flowability of a material is influenced by a number of factors. These include bulk density, particle size distribution, particle shape and aspect ratio, inter-particulate forces and surface texture (Neumann, 1967). Several methods have been acknowledged to measure the flowability of powders, most of which rely on a table by which the measured property is related to a descriptive term for the flowability. Properties such as angle of repose, mean avalanche time (Aeroflow®; Kaye, 1997), compressibility and minimum orifice diameter (Flodex®) are commonly used.

Throughout this study, the flow properties of α-lactose monohydrate carriers were qualitatively characterised by compressibility, as measured by their bulk and tapped densities (Carr, 1965). The bulk density was determined by pouring a sample of the powder into a suitable polypropylene measuring cylinder. The mass and volume of the powder were recorded and the bulk density calculated. The same sample was then placed on a jolting volumeter (J. Engelsmann, Ludwigshaven, Germany) and compressed by subjecting it to 100 standard taps. The tapped volume was recorded. The tapped volumes

after subsequent 100 tap sets were recorded until <2% volume change was observed across three consecutive readings. The tapped density was calculated from the tapped volume measurement and Carr's compressibility index (CCI) was calculated from:

Equation 2.5

$$CCI(\%) = 100\frac{\rho_t - \rho_b}{\rho_t}$$

where ρ_b is the bulk density and ρ_t is the tapped density.

2.2.6 Dynamic vapour sorption

To investigate the influence of storage at various environmental conditions on the aerosolisation performance of various lactose formulations, the susceptibility of different lactose samples for water vapour adsorption was challenged using the dynamic vapour sorption (DVS). The influence of humidity on water sorption and subsequent de-sorption of α-lactose monohydrate samples was determined gravimetrically over a range of controlled relative humidities. In simple terms, the DVS is based on an ultra-sensitive Cahn microbalance. Thus, the microbalance can accurately determine changes in mass of the sample with varying partial water vapour pressures. A sample and reference pans are continually perfused with nitrogen gas with a pre-set partial pressure of the solvent vapour probe in question. The instrument is housed in a temperature controlled chamber (5-85°C) and required humidities are generated by mixing dry and saturated vapour gases using a mass flow controller before passing over the sample and reference holders (Buckton and Darcy, 1995).

Moisture sorption of α-lactose monohydrate samples was determined using a DVS (DVS-1-system, Surface measurement systems Ltd., London, UK). Approximately 50 mg of sample was weighed into the sample cell and

subjected to a 0-90% RH cycle, over 10% RH increments. Equilibration moisture content at each humidity was determined when the change in mass in relation to the recording time (*dm/dt*) was 0.0002% min^{-1}.

2.2.7 Dry powder inhaler formulations

2.2.7.1 Preparation of powder formulations

Lactochem® α-lactose monohydrate was sieve fractioned for 20 minutes using a sieve shaker (Endecotts sieve and sieve shaker, London, UK). A test sieve with an aperture width of 90 µm was placed over a test sieve with an aperture width of 63 µm. The retrieved 63-90 µm sieve fractioned lactose was subsequently blended with micronised salbutamol sulphate (median diameter, d_{50} 4.79 µm) in a ratio of 67.5:1 w/w. The micronised salbutamol sulphate was sieved through a test sieve with an aperture width of 500 µm to break up any agglomerates formed during storage. The blending procedure involved initially adding a mass of lactose, approximately double the amount of salbutamol sulphate to be incorporated in the powder blend into a stoppered sample glass tube. The total mass of salbutamol sulphate was then directly weighed on top of the layer of lactose. The glass tube was stoppered and placed on a Whirlymixer (Fisons Scientific Equipment, Loughborough, UK) in a slanting position at 45° and mixed at maximum speed for 50 seconds. The blend was further diluted by the addition of lactose. The amount of lactose was equivalent to the total amount of drug and lactose in the glass tube. The glass tube was stoppered and mixed *via* the Whirlymixer, using the settings described above. The geometric addition of the lactose and mixing were repeated until all the lactose had been incorporated into the blend. The stoppered glass tube was then placed in a Turbula mixer and mixed at 46 $rev.min^{-1}$ for a further 30 minutes. Powder blends were stored in a controlled environment of 44% relative humidity until required. The controlled environment of the ambient 44% relative humidity

was obtained *via* the use of a saturated solution of potassium carbonate which was placed in a tightly sealed container (O'Brien, 1948).

2.2.7.2 Drug content uniformity and capsule filling

Ten samples of approximately 30.0 ± 2 mg of the powder blend (equivalent to the amount in the final dosage capsule), were randomly taken from various positions from the final blend which was evenly spread over a clean surface. The concentration of salbutamol sulphate was measured by either fluorescence spectrophotometry or high performance liquid chromatography. These are described in detail in section 2.2.7.4. The ratio of salbutamol sulphate to lactose of each sample was determined, and an acceptable dose uniformity of the mixture was when the relative standard deviation RSD% was less than 6.0.

Hard gelatine capsules (Size 3) were filled with 30.0 ± 2 mg of the powder mixture, such that each capsule contained a nominal dose of approximately 400 ± 30 µg of salbutamol sulphate. The filling was performed manually and filled capsules were stored in a controlled environment of 44% relative humidity until further required.

2.2.7.3 Formulation performance analysis

One of the most significant issues related to formulation development of DPI formulations is obtaining adequate deep lung delivery of the active pharmaceutical ingredient. As previously discussed, one of the most critical physical property which governs the behaviour of aerosolised particles when exposed to an air flow is particle size. It is important to note, however, that in case of drug particles for inhalation, the aerodynamic diameter is usually employed to characterise particle size, rather than their physical dimensions.

Since inertial impaction is the most commonly applied mechanism through which the aerodynamic particle size of aerosols is determined, inertial impactors were efficiently designed to represent the size fraction of airborne particles. Thus, enabling valuable *in vitro* examination of aerosolisation performance of tested formulations. In simple terms, a single stage impactor comprises a jet or nozzle plate containing an orifice of a defined diameter that is located at a fixed distance from a flat horizontal collection surface. Particle separation and sizing is achieved by the successive increase in the velocity of the air stream as it passes through the progressively reduced orifices of the nozzle plates. Whilst the incoming particles are travelling along the streamline, they are subjected to two forces. The first one is the momentum built up as they travel along the streamline, and the second one is the friction force with the surrounding air stream at the point of changing the direction of the laminar flow. The later force would drive the particles to accelerate in the new direction of air flow. That is to say, an airborne particle will continue to move in the original direction of flow until it loses inertia, it will then 'relax' into the new direction of flow. The deposition of particles onto the collection surface placed in the path of the original direction of air stream is dependent on particle diameter and density, velocity and viscosity of air stream, as well as the diameter of the jet orifice through which the air stream flows (Marple, 1970; Mitchell and Nagel, 2004).

Two different systems were employed throughout the study in analysing aerosolisation efficiency of the prepared DPI formulaitons. Both systems are based on impinger systems, that is, the deposition of drug particles within the *in vitro* apparatus is *via* impingement onto a liquid surface rather than impaction onto a solid surface. The desired particle cut-off size of each specific stage of an impinger/impactor is a complex relationship between the inertial and drag forces in an air flow, and is critically related to the airflow rate being drawn through the apparatus.

Twin-stage impinger

The twin-stage liquid impinger (TSI) apparatus was the first device to assess pulmonary drug delivery through inertial impaction which was implemented by the British Pharmacopoeia (BP) (British Pharmacopoeia Commission, 1993). The TSI is used for initial screening of inhalation devices and formulations, since it does not provide size distribution data but rather divides an aerosol into a coarse oropharyngeal fraction and a fine pulmonary fraction (Hallworth and Westmoreland, 1987). A representative TSI apparatus (Copley Scientific Ltd., Nottingham, UK) is shown in Figure 2.3.

The TSI apparatus contains two stages and a representative throat. The first stage contains 7 ml of solution, while the 2nd stage has 30 ml of solution. The TSI was operated at 60 L.min^{-1} (± 2 L.min^{-1}) to produce a cut-off mass median aerodynamic diameter of 6.4 µm between the two stages. The cut-off diameter of 6.4 µm is defined by the jet diameter of stage 1, which was measured as 13.84 mm. Dry powder formulations were aerosolised from a Cyclohaler® device (Novartis, Surrey, UK). Each capsule was tested at 60 L.min^{-1} for 5 seconds, given a total inhalation volume of 5L. Flow rate was calibrated using a flowmeter (SCR2, Glass Precision, Eng. Ltd., UK). A vacuum pump achieved the required flow rate through the TSI. The pressure drop across the apparatus was adjusted by a needle valve flow regulator (solenoid valve timer) (Copley Scientific Ltd., Nottingham, UK). The flow rate achieved through the device was adapted according to the inner resistance of the inhaler device. The pressure drop across the inhaler, established by a 60 L.min^{-1} flow rate, was set to 4.0 kPa in accordance with the USP (US Pharmacopoeia, 1992).

The capsule to be tested was placed in the Cyclohaler®, which had been specifically fitted with a moulded rubber mouthpiece for attachment to the throat piece of the TSI. Upon achieving of an air tight seal, the vacuum pump was switched on. The pump was allowed to run for 4 seconds prior to and post solenoid-valve actuation, to allow the pump to equilibrate. The deposition test was repeated for each drug-lactose blend for a minimum of

five capsules. The concentration of drug in the stages of the assembly was determined by either fluorescence spectroscopy or high performance liquid chromatography.

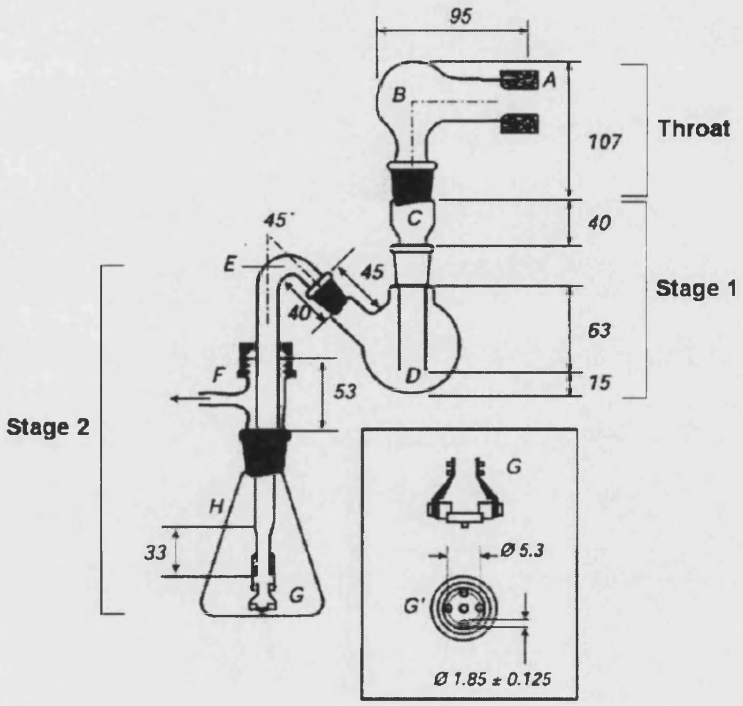

Figure 2.3. Schematic representation of a twin-stage liquid impinger, adapted from the British Pharmacopoeia, 2001, Volume II.

Multi-stage liquid impinger

The multi-stage liquid impinger (MSLI) apparatus, unlike the TSI, was developed to enable the determination of an aerodynamic particle size distribution of an aerosol, which is considered critical in respect of the deposition of particles *in vivo*. It also offers another advantage which lies in the fact that it is calibrated to measure the particle size of an aerosol at flow rates ranging from 30 to 100 $L.min^{-1}$. A representative MSLI apparatus (Copley Scientific Ltd., Nottingham, UK) is shown in Figure 2.4.

The impaction surfaces of the MSLI consist of sintered-glass discs immersed in the liquid (dilution solution). The MSLI apparatus consists of a throat, four classifying stages into which 20 ml of the dilution solution are introduced such that the entire surfaces of all the glass plates are wetted through capillary action. And a fifth dismantled stage on top of which a fiber glass filter paper is placed. Upon achieving an air tight seal, single capsules containing the nominal dose of 400 ± 30 µg of salbutamol sulphate were actuated *via* a Cyclohaler® (Novartis, Surrey, UK) at 60 $L.min^{-1}$ for 5 seconds. The device is specifically fitted with a moulded rubber mouthpiece for attachment to the throat piece of the liquid impinger. The flow rate was adjusted using a rotary vein pump and solenoid-valve (Copley Scientific Ltd., Nottingham, UK) and calibrated using a reference flowmeter (SCR2, Glass Precision, Eng. Ltd., UK). The pressure drop across the inhaler, established by a 60 $L.min^{-1}$ flow rate, was set to 4.0 kPa in accordance with the USP (US Pharmacopoeia, 1992). The pump was allowed to run for 4 seconds prior to and post solenoid-valve actuation, to allow the pump time to settle.

The concentration of drug in each stage was determined by rinsing with the dilution solution both the inner wall and the collection disc of the corresponding stage, followed by tilting and rotating the apparatus. The device, metal throat and the filter were also washed for drug extraction. The cut-off mass median aerodynamic diameters were 13.0, 6.8, 3.1 and 1.7 µm for stages 1, 2, 3 and 4, respectively, at 60 $L.min^{-1}$. Aerosol particles below 1.7 µm are collected on the filter stage. The respirable dose was calculated as the total mass of the drug found in the relevant impinger stages (stages 3, 4 and 5) representing an effective cut-off diameter ≤ 6.4 µm. The concentration of drug in the stages of the assembly was determined by either fluorescence spectroscopy or high performance liquid chromatography.

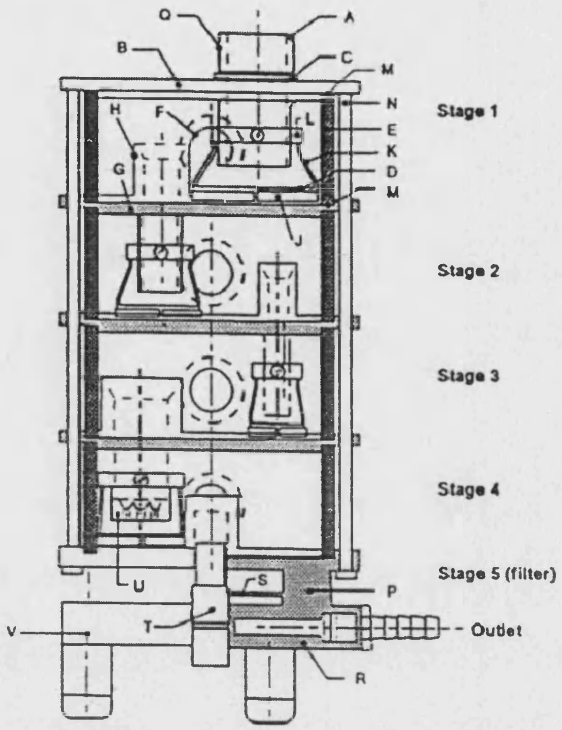

Figure 2.4. Schematic representation of a multi-stage liquid impinger, adapted from the British Pharmacopoeia, 2001, Volume II.

2.2.7.4 Drug analysis

The quantitative determination of drug concentrations in both blends content uniformity investigations and *in vitro* aerosolisation efficiency testing of formulations was carried by employing either fluorescence spectroscopy or high performance liquid chromatography (HPLC).

Fluorescence spectroscopy

Quantification of salbutamol sulphate content uniformity and *in vitro* deposition was determined by fluorescence spectroscopy (F-2000 Hitachi,

Ltd. Tokyo, Japan) with the following settings: excitation wavelength, 279nm; emission wavelength, 305nm. The mobile phase and wash solution used throughout was water. Linearity was determined between 1 and 10 μg ml^{-1}. Samples were diluted appropriately. α-Lactose monohydrate did not interfere with the salbutamol sulphate fluorescence response.

High performance liquid chromatography (HPLC)

Drug concentrations from both blend content uniformity testing and collected from various device components and TSI/MSLI sampling stages during *in vitro* studies were mainly analysed by high performance liquid chromatography (HPLC). Concisely, the HPLC operates by separating the organic compounds relative to their affinity for the solid phase in the HPLC column. The liquid chromatograph system was equipped with an autosampler (AS-950, Jasco, Tokyo, Japan), a pump (PU-980, Jasco, Tokyo, Japan), a column oven (CO-866, Jasco, Tokyo, Japan) and a UV/VIS-detector (UV-975, Jasco, Tokyo, Japan). Data were recorded and integrated using AZUR-OSIRIS Chromatography Software V 3.0. (Theix, France). Each value was determined by comparing the peak area of the sample to reference peaks of standard solutions of known concentrations. Each sample concentration was determined in duplicate.

A Sperisorb® 15 cm, 5 μm ODS1 column (Waters, Milford, MA, USA) was used for the analysis of salbutamol sulphate. Salbutamol sulphate was analysed using a mobile phase consisting of a 60:40 % v/v ratio of methanol to water mixture with 0.1% v/v of acetic acid. The HPLC pump was operated at a flow rate of 1.25 $ml.min^{-1}$ and a pressure of approximately 400 $kg.m^{2}$. The injection volume was 100 μl. The employed wavelength of the detector was 276 nm. The analysis run time was 4 minutes and the retention time for salbutamol sulphate was approximately 2 minutes.

Standard solutions of salbutamol sulphate were prepared prior to HPLC analysis. Duplicate stock standard solutions were prepared by accurately

weighing 10 mg aliquots of salbutamol sulphate into two 100 ml volumetric flasks and dissolving to volume with the mobile phase solution. Each stock solution was ultrasonicated for no more than 10 minutes to ensure dissolution, and then left to cool at room temperature and made up to the mark with the mobile phase solution. Working standard solutions were prepared by a carrying out a serial of dilutions of the stock standard solution using calibrated (class A) volumetric laboratory pipettes and the same mobile phase solution. A series of working standard solutions with concentrations comprised between 0.05 $\mu g.ml^{-1}$ and 10 $\mu g.ml^{-1}$ were subsequently prepared for salbutamol sulphate.

In order to correlate UV-absorbance response to drug concentration, the concentration-peak area response for salbutamol sulphate was investigated through linear regression analysis which is shown in Figure 2.5. A coefficient of determination (R^2) of 1 for salbutamol sulphate inferred a direct response between peak area and concentrations over the range of 0.05 $\mu g.ml^{-1}$ to 10 $\mu g.ml^{-1}$.

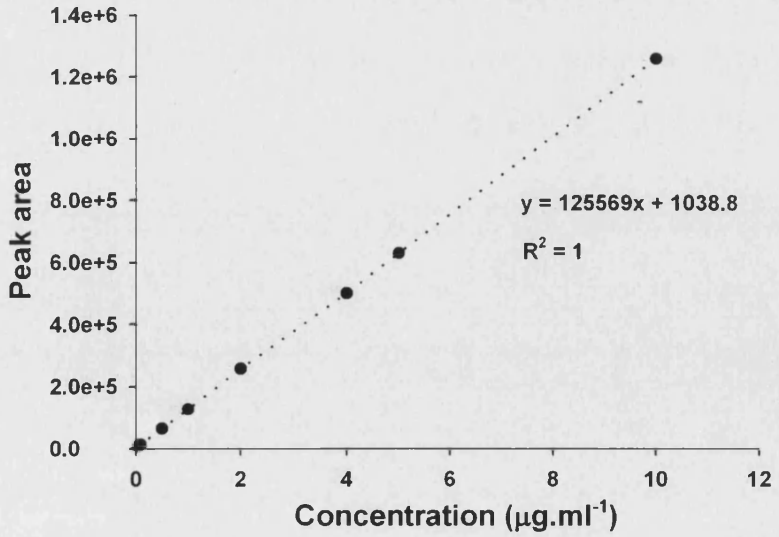

Figure 2.5. Peak area vs. concentration HPLC calibration plot of salbutamol sulphate.

2.2.8 Statistical analyses

The average and deviation of normal distribution populations were expressed as arithmetic mean ($\bar{x}$) and standard deviation (SD).

Arithmetic mean ($\bar{x}$) was calculated by:

Equation 2.6

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

The standard deviation (*SD*) of a pool of numbers was calculated using:

Equation 2.7

$$SD = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(\bar{x} - x_i)^2}$$

The percent relative standard deviation *RSD* (%) also known as the coefficient of variation was calculated by:

Equation 2.8

$$RSD(\%) = \frac{SD.100}{\bar{x}}$$

Statistical analyses between different populations were carried out by one-way ANOVA. A population was regarded as significantly different if the assumption of similarity was rejected with a probability greater than 95%. Comparison of the means was performed by Fisher's pair wise comparison.

Chapter 3

Novel temperature controlled surface etching of lactose particles for dry powder inhalation formulations

3.1 Introduction

The successful development of carrier based dry powder inhaler (DPI) formulations has been achieved through the design of ever increasingly elaborate devices and an improved understanding of the role of the interactions between the inhaler components, namely drug, carrier and device. Generally, a DPI is developed and optimised based on an iterative process of formulation preparation and testing. Excipient lactose particles, sourced from various suppliers, each exhibiting different physicochemical characteristics are invariably blended with micronised drugs, of various doses. The formulation is then studied using an *in vitro* aerodynamic particle size classifier, for example, a liquid impinger or a cascade impactor. The aerosolisation behaviour of the formulation is subsequently described in terms of the respirable fraction and emitted dose. The characteristic properties of the carrier, drug and processing conditions are then iterated to produce a formulation which exhibits a reproducible and acceptable dosage profile. This time consuming process has proved very successful by considering the plethora of products on the market which satisfy the stringent requirements of the relevant regulatory authorities (Smith and Parry-Billings,

2003). However, there are many questions surrounding the nature and effect of particle interactions in a DPI formulation and it is clear that one lactose or drug type or batch or device will not provide universally optimal DPI performance. One of the key concerns within the industry is the *inter* and *intra* batch performance of lactose and the properties and characteristics of batches of the micronised drug (Byron *et al.*, 1996). Indeed, it has been reported that inter-batch variations of lactose can influence formulation performance, especially for low dose drug formulations (Steckel *et al.*, 2004). This can, in part, be explained in terms of particle size variations and surface roughness and, more importantly, how accurately the particle descriptors characterise both the lactose and drug.

The relationships between particle size and its distribution, shape and roughness, and aerosolisation behaviour have been investigated for carrier based DPI formulations. There have been reports of the effect of lactose source and particle characteristics on the efficiency of DPI formulations (Kawashima *et al.*, 1998a; Larhrib *et al.*, 1999; Louey *et al.*, 2003). Recently, attention has focussed on the nature and role of the fine lactose particles on formulation performance and it has been suggested that the efficiency of a formulation can be optimised with a certain level of fines (Zeng *et al.*, 1998; Islam *et al.*, 2004a). However, the number of reports describing the relationship between the properties of the carrier and apparent formulation performance can be contradictory (Louey *et al.*, 2003).

In view of the difficulties in understanding, predicting and comparing the relationships between carrier properties and drug particle interactions, both drug and excipient have been further processed to produce modified surfaces/particle size distributions which offer more reproducible and improved performance. Such approaches may ultimately allow the production of carriers which exhibit improved batch to batch consistency and stability. A similar approach has been used for drugs where drugs, such as salbutamol sulphate, have been engineered by crystallisation (Larhrib *et al.*, 2003a) and by supercritical fluid technology (Shekunov *et al.*, 2003; Rehman *et al.*, 2004; Schiavone *et al.*, 2004; Young and Price, 2004).

The modification of lactose can be divided into three general areas; crystallisation, where lactose is crystallised from a lactose solution; solution phase processing, where lactose is exposed to a liquid media where partial dissolution/etching may occur; and dry processing, where lactose is 'treated' by, for example, co-processing in the absence of a liquid. These methodologies attempt to increase the aerosolisation of drug *via* geometric and morphological modifications. The relationships between lactose surface roughness, crystal aspect ratio and geometry and drug aerosolisation have been studied by crystallisation of lactose from water (Zeng *et al.*, 2000a), water/acetone (Larhrib *et al.*, 2003b) and carbopol/water/ethanol (Zeng *et al.*, 2001b; Larhrib *et al.*, 2003a). These investigations reported an increase in aerosolisation of salbutamol sulphate and terbutaline sulphate with decreasing lactose roughness compared to 'as supplied' lactose. This is in agreement with a recent study of the relationship between the surface roughness of sieved lactose and aerosolisation of terbutaline sulphate (Flament *et al.*, 2004). It has also been reported that increasing the surface roughness of lactose increases the emitted dose, but reduces the sub 6.4 μm respirable dose (Heng *et al.*, 2000). Solution phase processing of lactose has been achieved by treating lactose with various liquids. Aqueous alcohol has been used to partially dissolve surface asperities resulting in an increase in the aerosolisation of salbutamol sulphate (Iida *et al.*, 2003a). Lactose has also been treated by decantation with alcohol to remove lactose fines which resulted in a decrease in the aerosolisation of salmeterol xinofoate which was restored after the re-addition of fines (Islam *et al.*, 2004a). Additionally, lactose has been treated or 'smoothed' with a suspension of magnesium stearate in water/ethanol under high shear which resulted in an improvement in the performance and efficiency of a DPI formulation (Young *et al.*, 2002). Such processes may result in some degree of dissolution or etching of lactose and the dissolution of fines from the crystal surfaces and it was not clear if part of any apparent improvement in the aerosolisation performance was due to modification of the particle size or the distribution of fines, since a reduction in carrier particle size has been reported to improve drug aerosolisation (Steckel and Müller, 1997b; Louey *et al.*, 2003). Non liquid based treatment of lactose has been achieved by dry blending with leucine to

improve functionality (Staniforth, 1996a; Lucas *et al.*, 1999). It can be also argued that addition of lactose in the form of fine particulates to coarse lactose is another form of dry co-processing.

One problem with these types of investigations is that "modification" of a lactose sample invariably results in a change in the particle size and surface characteristics. It is practically impossible to absolutely classify particles in terms of their particle size distribution and surface roughness. This has obvious implications for both carrier and drug and explains the difficulties for quantitative comparisons of formulation performance. The relationship between modification and particle size distribution is further complicated by the possible methodology dependence of apparent particle size descriptors (Larhrib *et al.*, 1999). The previously described reports suggest that for single dose devices, a decrease in lactose surface roughness together with an optimal level of fines should result in an improved lactose carrier performance, which again will be drug and device dependent. Obviously, the production of a lactose carrier which would facilitate classification would be attractive since it would reduce the effect of any possible batch to batch differences. As part of the efforts undertaken to develop multi-purpose DPI lactose carriers, this study describes an investigation into a novel particle surface etching process developed to evaluate the influence of carrier modification on the delivery of salbutamol sulphate from a model carrier based DPI formulation.

3.2 Materials

α-Lactose monohydrate (Lactochem® crystals) was supplied by Borculo Whey (Chester, UK). The lactose was vibrated through a nest of sieves to obtain a 63-90 μm sieve fraction, which was used throughout the study. Micronised salbutamol sulphate was supplied by Aventis Pharma (Holmes Chapel, UK). Water used was purified by reverse osmosis (MilliQ, Molsheim,

France). All solvents used throughout the study were supplied by BDH (Poole, Dorset, UK) and were of at least of analytical grade.

3.3 Methods

3.3.1 Solubility profile of α-lactose monohydrate

An accurate temperature-water solubility profile for α-lactose monohydrate was determined. A supersaturated solution of lactose in water was prepared by continued addition of lactose to 20 ml of water. The solution was placed in a controlled temperature water bath at differing temperatures (Haake, DC5, Fisons Scientific Equipment, Loughborough, UK) and vigorously shaken for 48 hours. Each equilibrated sample was rapidly filtered under vacuum through a 0.2 μm filter. The recovered solution was weighed and dried in an oven at 50°C. The resulting dry mass was re-weighed and solubility calculated. Solubility of α-lactose monohydrate was determined between 20°C and 45°C at 5°C intervals.

3.3.2 Preparation of surface etched α-lactose monohydrate

The surface etching of lactose was achieved as follows. A saturated solution of α-lactose monohydrate, in water, was prepared and continually stirred at a constant temperature of 25°C. Temperature within the vessel was controlled to within 0.1°C *via* a refrigerated controlled water bath (Haake, DC5, Fisons Scientific Equipment, Loughborough, UK). A known volume of the saturated solution (100 ml) was removed from the vessel, filtered and transferred to a dissolution cell, which was maintained at the saturation temperature. A pre-determined amount of 63-90 μm sieved α-lactose monohydrate ($M_{initial}$), e.g. 50 g, was added to the saturated solution in the dissolution cell. Surface controlled etching of the lactose particles surfaces was achieved by either

ramping the temperature within the dissolution vessel at a controlled rate (0.1-0.5°C.min^{-1}) or directly increasing to the etching temperature. The difference between the starting, 25°C, and the final temperature is referred to as the etching temperature, while the degree of etching can be directly quantified *via* utilising the following expression for undersaturation (σ):

Equation 3.1

$$\%\sigma_{under} = -\frac{C_T - C_S}{C_S} \times 100$$

Where C_T is the solubility of lactose at the etching temperature and C_s is the solubility at the saturation temperature.

With prior knowledge of the temperature dependence of the solubility of α-lactose monohydrate, the undersaturation conditions and therefore the degree of or etching ($\%M_{etched}$), of the sieve fractioned crystals could be directly quantified. If the mass of added sieved lactose is greater than the 'dissolution capacity' of the liquid, the percentage dissolved lactose, in terms of mass of the sieved lactose added, can be accordingly quantified *via* the following expression:

Equation 3.2

$$\%M_{etched} = \Delta C_T(\%)V_L(ml)/M_{initial}(g)$$
$$\text{for } M_{initial}(g) \geq \Delta C_T(\%)V_L(ml)/100$$

Where ΔC_T is the difference in the solubility of lactose in the solution at the saturation temperature and at the etching temperature. $M_{initial}$ is the original mass of 63-90 μm sieve fractioned lactose added to a known volume of the saturated lactose solution (V_L). $\Delta C_T(\%)V_L/100$ is the 'dissolution capacity'.

3.3.3 Particle size analysis

The particle size distributions of the untreated and surface etched lactose samples were determined by laser light scattering using a Malvern Mastersizer X (Malvern Instruments Ltd, Malvern, Worcs, UK). The instrument is equipped with a magnetically stirred sample cell with a capacity of approximately 20 ml. A 0.1% w/w lecithin/cyclohexane solution was used as a sample dispersant. To deagglomerate cohesive powders, each sample was analysed prior and post 5 minutes ultrasonication, hence, ensuring a more accurate assessment of the fine particulate content. A 300 mm lens was used, as it allows the detection of particle size in the range of 1.2 to 600 µm. Approximately 1 mg of the material was suspended in the dispersant and continuously stirred in order to obtain an obscuration level of 20-30%. The particle size distribution was calculated and represented as a volume distribution. All samples were prepared and analysed in triplicate.

3.3.4 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to characterise particle shape and surface morphology of powder samples. Representative powder samples were sprinkled on adhesive black carbon tabs, which were pre-mounted on aluminium stubs. A thin film of gold was vaporised onto the sample surface using a sputter coater (Model S150B, Edwards High Vacuum, Sussex, UK). Samples were then examined using a JEOL 6310 SEM (Japanese Electron Optics Ltd, Tokyo, Japan) at 10 KeV.

3.3.5 Specific surface area

The surface area of the untreated and surface etched lactose was determined by a BET adsorption method (Gemini, Micromeritics Ltd, USA)

using nitrogen and helium gas. Samples were dried under a stream of dry nitrogen at 40°C for 16-20 hours prior to analysis.

3.3.6 Powder bulk and tapped density and flow

Powder flowability was represented by bulk and tap density measurements. The bulk and tap density of each lactose sample was obtained by adding 50-100 ml of powder sample into a 100 ml measuring cylinder. The initial volume and mass was recorded and then sample was subjected to 100 standard taps using a jolting volumeter (J. Engelsmann, Ludwigshaven, Germany), and the tapped volume recorded. The volume after subsequent 100 tap sets were recorded until <2% volume change was observed across three consecutive readings. Bulk, tap densities and Carr's compressibility index were calculated.

3.3.7 X-ray diffraction

X-ray diffraction of untreated and surface etched lactose samples was obtained using an X-ray powder diffraction system (D5000, Bruker AXS, Cheshire, UK). Settings were as follows; 2° to 39.960° 2θ, step size 0.036° 2θ, step time 0.5 seconds, temperature 25°C.

3.3.8 In vitro aerosolisation studies

The relationship between carrier surface etching and the *in vitro* performance of drug-carrier blends was investigated using a twin stage impinger (TSI). Micronised salbutamol sulphate, the most commonly used short acting β_2-antagonist used in asthma therapy, was used as a model drug. The micronised salbutamol sulphate (median diameter, d_{50} 4.79 µm) was

geometrically blended with the untreated and surface etched lactose samples at a ratio of 67.5:1 w/w using a Whirlymixer (Fisons Scientific Equipment, Loughborough, UK) in 50 second pulses.

Upon completion of the geometric mixing, the blend was placed in a Turbula (Bachofen, Basel, Switzerland) and blended at 46 rev.min^{-1} for 30 minutes. The final blend was stored in a tightly sealed container with a saturated salt solution of potassium carbonate, which produced a relative humidity of 44% RH at 25°C (O'Brien, 1948). Prior to *in vitro* studies, content uniformity were investigated by analysing the drug content in 30.0 ± 2 mg samples (n=10) for each blend.

Quantification of salbutamol sulphate content uniformity and *in vitro* deposition was determined by fluorescence spectroscopy (F-2000 Hitachi, Ltd. Tokyo, Japan) with the following settings: excitation wavelength, 279nm; emission wavelength, 305nm. The mobile phase and wash solution used throughout was water; Linearity was determined between 1 and 10 µg.ml^{-1}. Samples were diluted appropriately. α-Lactose monohydrate was shown not to not interfere with the salbutamol sulphate fluorescence response (Young and Price, 2004), when using water as a dilution solvent.

Hard gelatine capsules (Size 3) were filled with 33 ± 4 mg of powder blend, such that each capsule contained a nominal dose of 482 ± 58 µg of salbutamol sulphate. The aerosolisation properties of the salbutamol sulphate-lactose formulations were investigated using an apparatus A (British Pharmacopoeia), the twin stage impinger (TSI) (Copley Instruments Ltd, Nottingham, UK). The first stage contained 7 ml of water and the second stage contained 30 ml of water. At 60 L.min^{-1}, the cut-off mass median aerodynamic diameter was 6.4 µm between the two stages (Hallworth and Westmoreland, 1987). Each capsule was tested *via* actuation for 5 seconds using the Cyclohaler® (Novartis, Surrey, UK) single shot dry powder inhaler device. The pressure drop across the apparatus was calibrated using a micromanometer (model FC012, Furness controls, Bexhill, UK). The flow rate was achieved using a rotary vein pump and solenoid-valve (Copley Scientific,

Nottingham, UK), and adjusted using a calibrated flow meter. The pump was allowed to run for 4 seconds prior to and post solenoid-valve actuation, to allow the pump time to settle. The volume of air drawn through the *in vitro* apparatus was set at 5L. The concentration of drug in each stage/device was determined by washing the content of each stage into a volumetric flask with water before chemical analysis with the pre-calibrated fluorescence spectrophotometer (F-2000 Hitachi, Ltd. Tokyo, Japan). Each *in vitro* test was repeated for each drug-lactose blend for a minimum of five capsules.

The aerosolisation characteristics were described as: loaded dose (LD), drug recovered from the capsule, mouthpiece, throat and stages 1 and 2; emitted dose (ED), drug emitted from the device into mouthpiece adapter, throat and stages 1 and 2; fine particle dose (FPD), drug in stage 2 of the TSI; fine particle fraction (FPF), percentage of FPD to LD; delivered dose, percentage ED to LD.

3.4 Results and discussion

3.4.1 Solubility profile of α-lactose monohydrate

α-Lactose monohydrate is considered to be freely soluble in water (British Pharmacopoeia), exhibiting a solubility of 1 g in 4.63 ml water at 25°C (Handbook of Pharmaceutical Excipients). Accurate values were determined and were found to be fitting those obtained from literature (Jelen and Coulter, 1973a; Thurlby and Sitnai, 1976). The experimentally determined water solubility values of lactose at varying temperatures are presented in Table 3.1.

Temperature (°C)	Solubility (g/100ml)
20	19.6705
25	21.6875
30	25.001
35	28.241
40	32.1395
45	36.8035

Table 3.1. Aqueous solubility values of α-lactose monohydrate with varying temperatures.

The solubility versus temperature profile was fitted with a 3rd order polynomial equation. The relationship exhibited a correlation coefficient, R^2, of 0.999. The influence of temperature on the solubility profile of α-lactose monohydrate is shown in Figure 3.1. The solubility dependency can be modelled by the following expression:

$$S(T) = 19.892 - 0.2937T + 0.0138T^2 + 0.00003T^3$$

Where S(T) is the solubility of lactose in water (g/100ml) at a specific temperature and T is the temperature of the solution in °C.

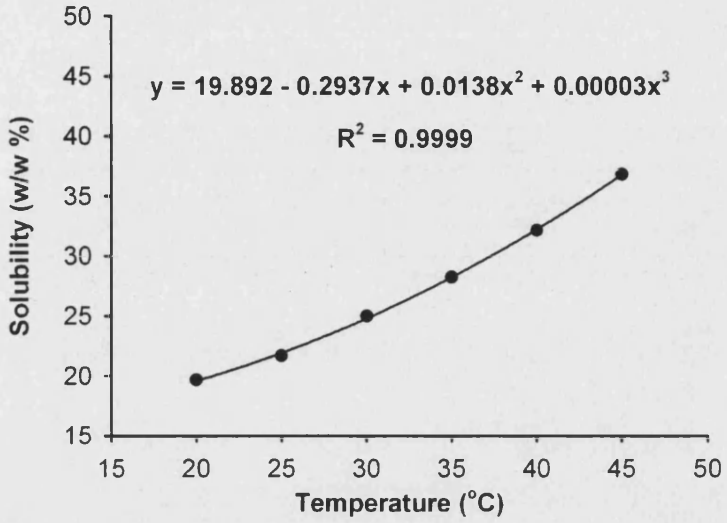

Figure 3.1. Aqueous solubility profile of α-lactose monohydrate.

3.4.2 Surface etched α-lactose monohydrate

The variable etching conditions for surface modification of the lactose excipient particles were determined through knowledge of the solubility versus temperature profile. Once the saturation temperature, volume of the saturated solution and the mass of 63-90 μm sieve fractioned α-lactose monohydrate added to be etched are known, the temperature conditions for the etching process can be accurately determined and controlled. Table 3.2 shows the percent undersaturation (%σ) and accordingly the percent mass etched ($\%M_{etched}$) of 50 grams of sieve fractioned α-lactose monohydrate as a function of temperature difference (ΔT). The lactose particles to be etched were added to 100ml of solution that was saturated at 25°C.

Temperature difference	% Undersaturation	% Of initial mass etched
$\Delta T = T_{etch} - T_{sat}$ (°C)	$\%\sigma = -(\{C_T - C_s\} / C_s) \times 100$	$\%M_{etched} = (\{\Delta C_T \times V_L\} / M_{initial}) \times 100$
5	-12.15	5.27
10	-28.47	12.35
15	-48.96	21.24
20	-73.63	31.94
25	-102.461	44.45

Table 3.2. Percent undersaturation and percent mass etched of α-lactose monohydrate at varying temperature differences.

This relationship is industrially scalable and requires only a limited number of additional steps to current industrial production and processing. It should be noted that unlike crystal growth at conditions of low supersaturation, the kinetics of dissolution are rapid. Furthermore, once saturation conditions have been achieved at the etching temperature, further dissolution and crystal growth are inhibited.

3.4.3 Physical characterisation

The effect of surface etching of α-lactose monohydrate on the aerosolisation of salbutamol sulphate was investigated. Prior to the aerosolisation studies, the untreated and surface etched lactose were initially characterised in terms of particles size, morphology, surface area, flowability and crystallinity.

3.4.3.1 Particle size analysis

A graphical representation of the particle size and cumulative undersize distributions of untreated and various % mass etched α-lactose monohydrate are shown in Figures 3.2, 3.3, 3.4 and 3.5. Each powder sample was analysed both with and without sonication. Untreated commercial α-lactose

monohydrate was found to have a considerable level of fine particles, as observed upon sonication of the lactose sample Figure 3.2 B. Figures 3.3 and 3.4 clearly indicate that no significant variation was observed between unsonicated and sonicated samples of 5% and 21% surface etched lactose, respectively. Comparison of the cumulative undersize data between untreated and all surface etched lactose samples is shown in Figure 3.5. The data highlights the removal of fines from the commercial lactose sample. No marked differences were observed between the different % mass etched lactose samples. The data suggested that the surface etching process modified the particle size distribution to a larger d_{50} and a reduction in the number of fines. These results also suggest that the temperature controlled surface etching process did not alter the overall particle size distribution of lactose excipient. The surface area of the surface etched lactoses, shown in Table 3.3, remained unchanged.

As previously stated, any apparent change in functionality may, in part, be a consequence of this removal of fines. The presence and level of fines in lactose carriers is widely reported as a crucial factor in DPI performance (Zeng *et al.*, 1998; Zeng *et al.*, 1999; Louey *et al.*, 2003; Islam *et al.*, 2004a).

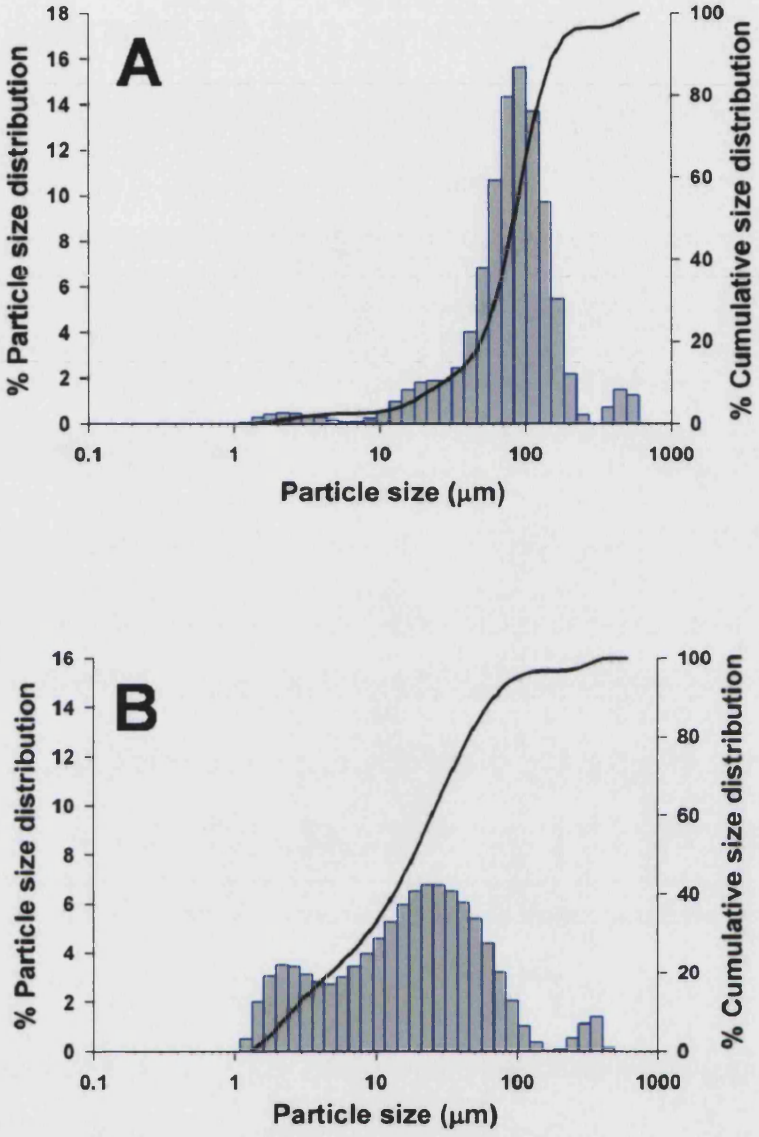

Figure 3.2. Cumulative particle size distribution of untreated α-lactose monohydrate before (A) and following 5 minutes sonication (B).

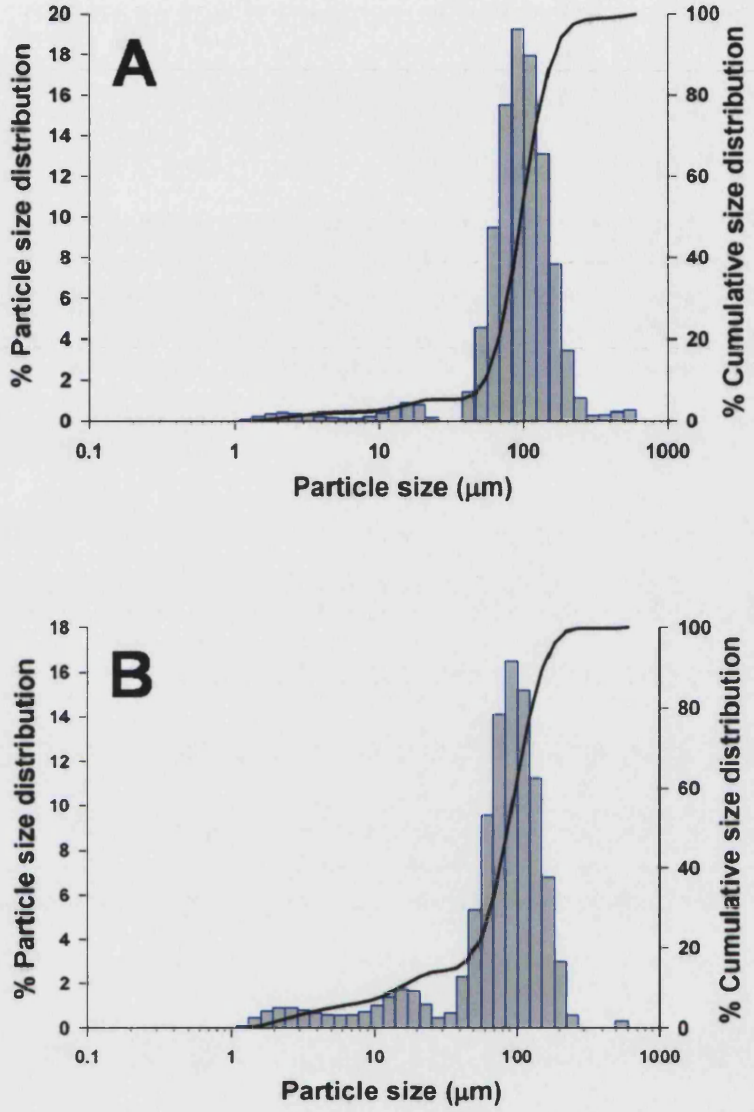

Figure 3.3. Cumulative particle size distribution of 5% etched α-lactose monohydrate before (A) and following 5 minutes sonication (B).

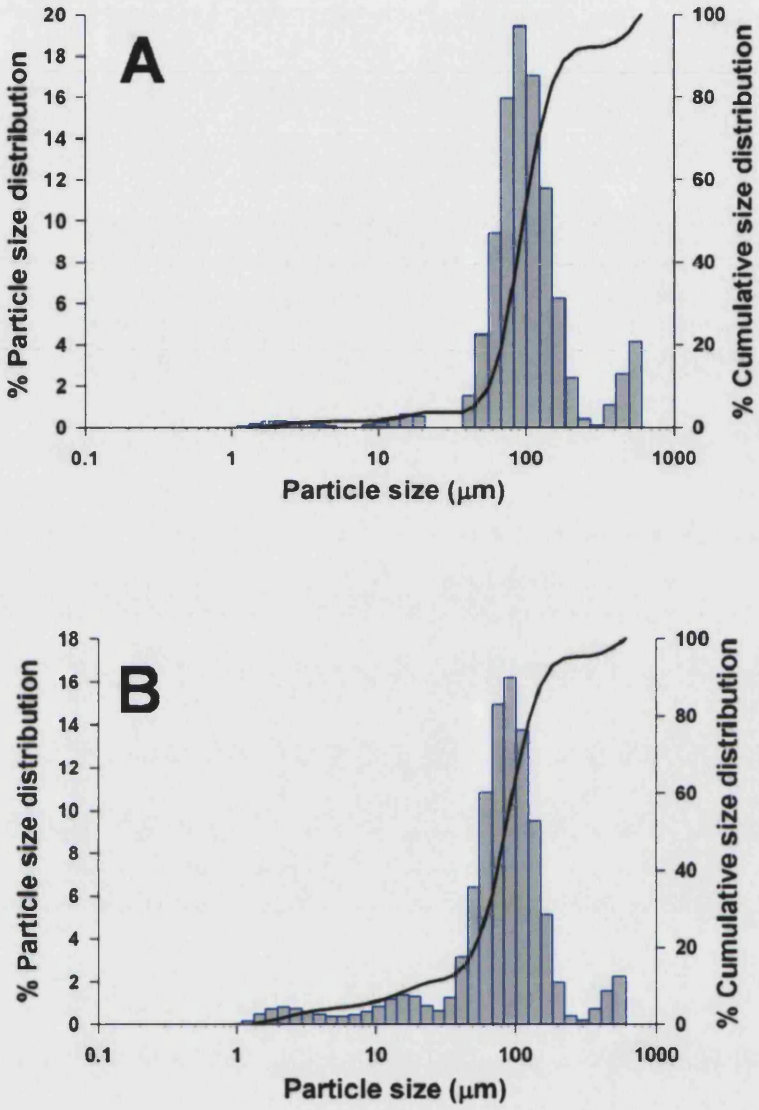

Figure 3.4. Cumulative particle size distribution of 21% etched α-lactose monohydrate before (A) and following 5 minutes sonication (B).

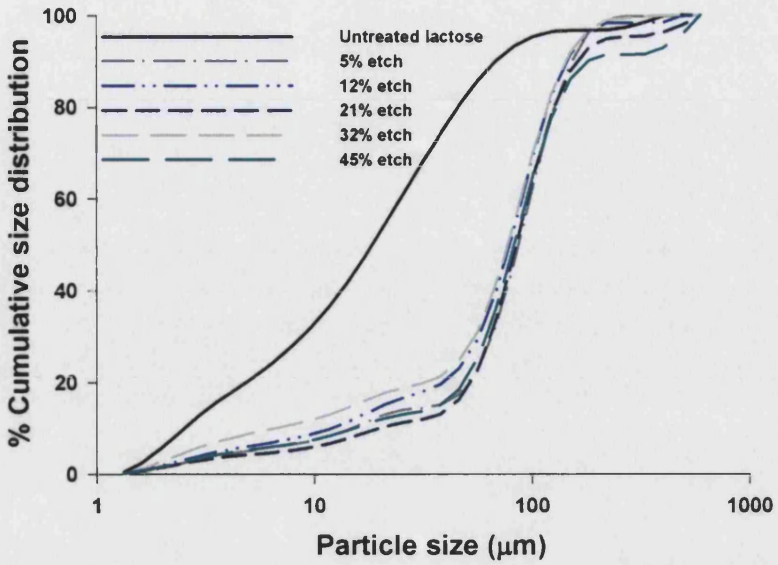

Figure 3.5. Cumulative undersize distribution of untreated and various degrees of % mass etched α-lactose monohydrate.

3.4.3.2 Scanning electron microscopy

Figure 3.6 shows SEM photomicrographs of the surface topography of as supplied commercial grade α-lactose monohydrate. Untreated lactose micrographs indicated a considerable degree of surface roughness with significant variations in surface properties of the lactose particles. In addition, there was a significant level of fine particulates of lactose present on the surfaces of the untreated sample, in strong agreement with particle size analysis data.

The specific influence of etching conditions on the surface texture of lactose was also examined by scanning electron microscopy. Representative scanning electron micrographs of controllably surface etched crystals of α-lactose monohydrate with increasing the degree of etching are shown in Figures 3.7, 3.8, 3.9, 3.10 and 3.11. Distinct differences are exhibited for each particular surface treatment.

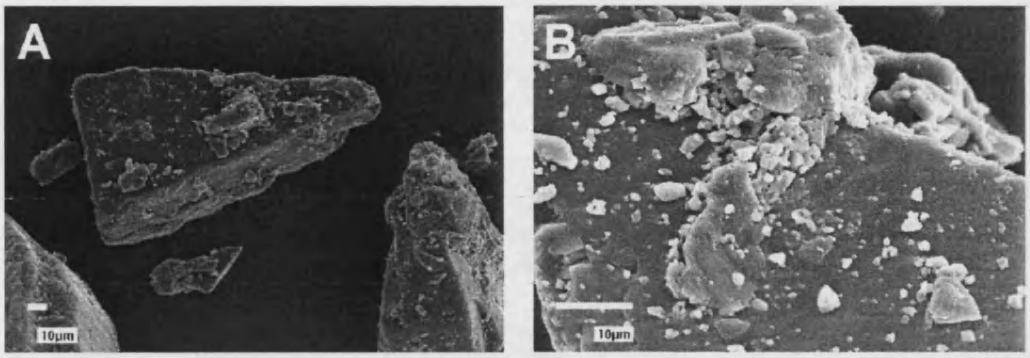

Figure 3.6. Representative scanning electron photomicrographs of untreated α-lactose monohydrate crystals at X500 (A) and X2000 (B) magnifications.

SEM photomicrographs of 5% surface etched α-lactose monohydrate particles, shown in Figure 3.7, clearly demonstrate an observable change in surface topography. Most noticeably, there is a visual decrease in the level of fines, accompanied by a dramatic decrease in the apparent surface roughness of lactose particles upon processing when compared to untreated lactose particles.

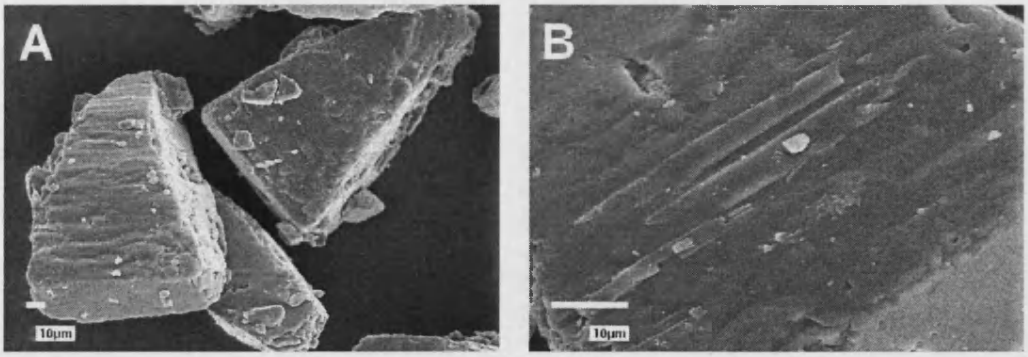

Figure 3.7. Representative scanning electron photomicrographs of 5% surface etched α-lactose monohydrate crystals at X500 (A) and X2000 (B) magnifications.

Increasing the undersaturation conditions of the lactose solution to 12% indicated complete dissolution of the intrinsic lactose fines, as shown in Figure 3.8. This increasing in etching conditions further increased the degree of surface smoothness, without modifying the macroscopic morphology (i.e. particle size and shape) of the lactose crystal.

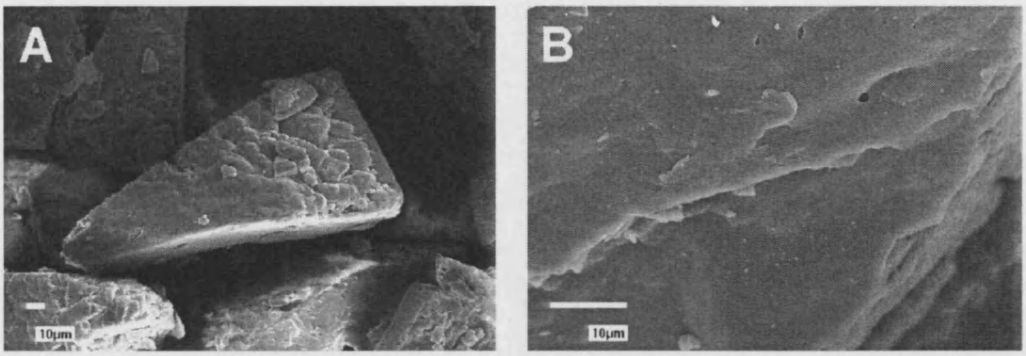

Figure 3.8. Representative scanning electron photomicrographs of 12% surface etched α-lactose monohydrate crystals at X500 (A) and X2000 (B) magnifications.

Further increasing the degree of undersaturation to 21% indicated complete elimination of irregularities, yet maintaining good binding sites for drug particles Figure 3.9.

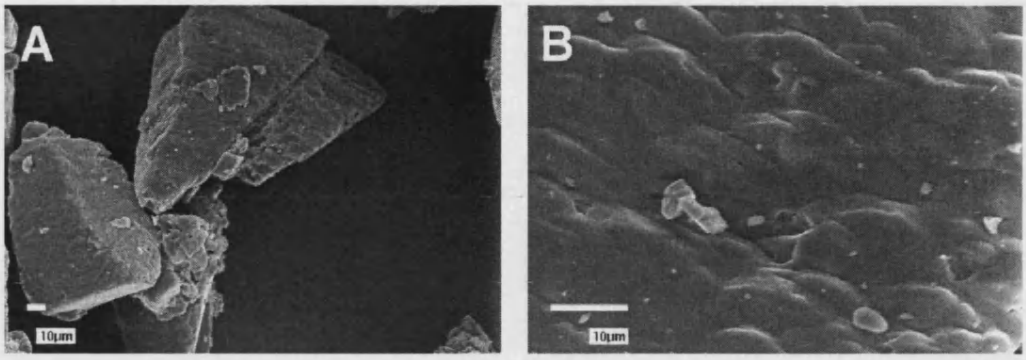

Figure 3.9. Representative scanning electron photomicrographs of 21% surface etched α-lactose monohydrate crystals at X500 (A) and X2000 (B) magnifications.

Since the apparent degree of surface dissolution increased with increasing the dissolution temperature, etching of α-lactose monohydrate particles surfaces at a degree of undersaturation exceeding 21% exhibited extremely smooth surface topography. However, noticeable alterations of crystal shape and morphology were observed in the representative SEM micrographs at 32% and 44% mass etched lactose particles Figures 3.10 and 3.11.

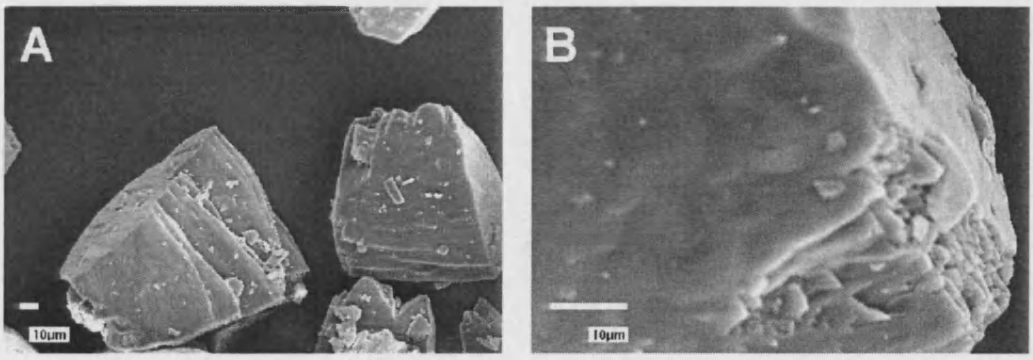

Figure 3.10. Representative scanning electron photomicrographs of 32% surface etched α-lactose monohydrate crystals at X500 (A) and X2000 (B) magnifications.

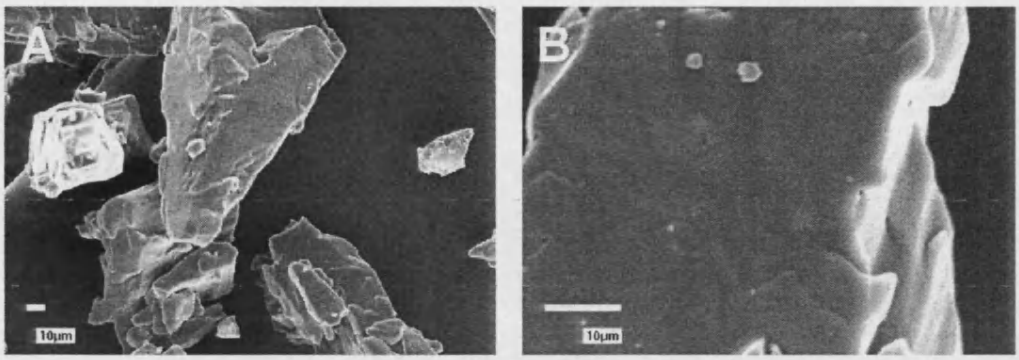

Figure 3.11. Representative scanning electron photomicrographs of 44% surface etched α-lactose monohydrate crystals at X500 (A) and X2000 (B) magnifications.

3.4.3.3 Specific surface area

Surface area measurements of unmodified and surface etched α-lactose monohydrate samples are shown in Table 3.3. It can be seen from Table 3.3 that all the samples exhibited similar (ANOVA $p<0.05$) ($n=3$) surfaces areas of approximately 0.11 m^2g^{-1} suggesting that etching does not have a dramatic effect on the bulk surface area of the processed lactose.

3.4.3.4 Powder bulk and tapped density and flow

The powder flow of untreated commercial grade and surface etched lactose, as represented by Carr's compressibility index, is shown in Table 3.3. In general, the blends exhibited similar (ANOVA $p<0.05$) Carr's compressibility indices of 21% ± 2 suggesting that the surface etching process did not dramatically change the flow properties of the powders. There were no significant differences between the bulk and tapped densities of the samples. Commercially available sieved lactoses of different mesh sizes (d_{50} 135 to 55 µm) also exhibit similar Carr's compressibility indices and powder densities (Handbook of Pharmaceutical Excipients).

	Etching temperature (°C)				
	Untreated	5	10	15	25
Mass of lactose dissolved (%)	-	5	12	21	44
Lactose surface area ($m^2 g^{-1}$)	0.13 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Carr's compressibility index (%)	19 ± 1	19 ± 1	24 ± 1	18 ± 1	24 ± 1

Table 3.3. Specific surface area and Carr's compressibility index values for untreated and various % mass etched α-lactose monohydrate samples.

3.4.3.5 X-ray diffraction

X-ray diffraction patterns for both untreated and 44% surface etched lactose are shown in Figure 3.12. No modification of the crystal structure upon surface etching was observed. Such observations are to be expected since the processing method does not induce crystal growth and α-lactose monohydrate is the most stable lactose form under such experimental conditions. The changes in the relative intensity of the diffraction lines are most likely due to the geometry of the crystals in the equipment and possibly related to the removal of the lactose fines.

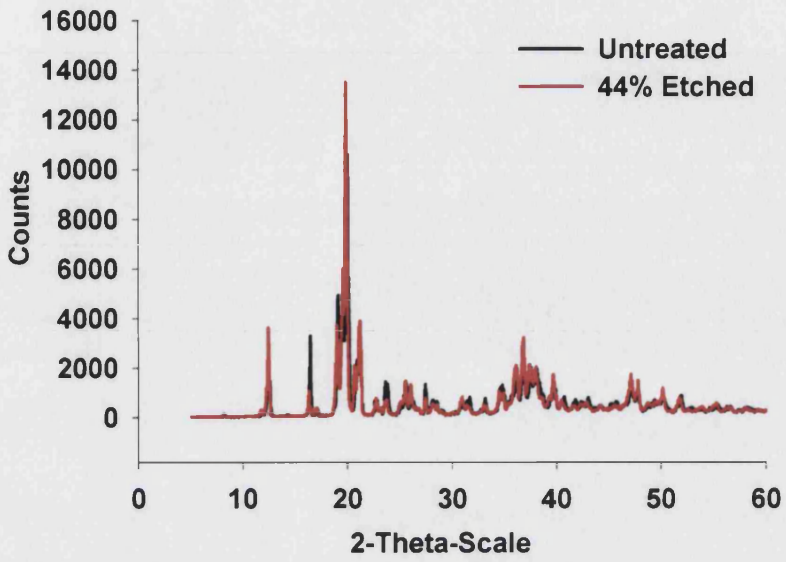

Figure 3.12. X-ray powder diffractogram for untreated and 44% surface etched α-lactose monohydrate samples.

3.4.4 In vitro aerosolisation studies

The relative standard deviation in the content uniformity of the salbutamol sulphate-lactose blends was less than 3%. The TSI aerosolisation efficiency of salbutamol sulphate, as represented by FPD and FPF, is summarised in Table 3.4 and shown graphically in Figures 3.13 and 3.14.

	Etching temperature (°C)					
	Untreated	5	10	15	20	25
Emitted dose (µg)	350.8 ± 25.6	288.1 ± 45.4	359.9 ± 147.9	389.0 ± 55.2	371.0 ± 16.6	435.3 ± 18.2
Delivered dose (%)	82.2 ± 4.2	82.7 ± 1.8	71.5 ± 21.7	82.2 ± 4.8	81.8 ± 1.6	84.6 ± 2.2
Fine particle dose (FPD) (µg)	56.1 ± 15.1	62.2 ± 31.6	83.5 ± 9.4	108.3 ± 47.2	88.4 ± 11.1	102.7 ± 9.5
Fine particle fraction (FPF) (%)	13.1 ± 3.3	17.2 ± 6.8	17.3 ± 3.1	22.6 ± 8.1	19.5 ± 2.0	20.0 ± 2.0

Table 3.4. Deposition profile of salbutamol sulphate (mean ± standard deviation) in a TSI after aerosolisation of untreated and % mass dissolved lactose formulations (Cyclohaler®, 60 $L.min^{-1}$).

No significant differences (ANOVA $p<0.05$) in either ED (451.8 ± 58.9 µg) and % delivered dose (~81%) were observed for all blends. The results, however, clearly indicated an increase in the deposition of the active salbutamol sulphate particles in the second stage of the TSI, upon modifying the surface texture of lactose particles. Particles deposited in stage 2, which has an effective cut off diameter of less than 6.4 µm, represent the fine particle dose (FPD). In comparison, analysis of the FPD and FPF suggested that surface etching at 35°C had a significant effect (>12%) on drug liberation (Fisher's pairwise analysis, $p<0.05$). However, a decrease in fine particle fraction with further increasing the etching conditions was also observed. This may be as a result of obtaining an extremely smooth surface at high etching temperatures exhibiting marked decrease in nanometer scale surface roughness, which may lead to an increase in the contact area between micron sized drug particles and the carrier surface. In such circumstances, the increase in contact area between drug and excipient may directly increase the force of adhesion and concomitantly the energy required to aerosolise the active particles upon inspiration. Given this effect, maintaining an optimum degree of carrier surface roughness is of great significance as schematically illustrated in Figure 3.15.

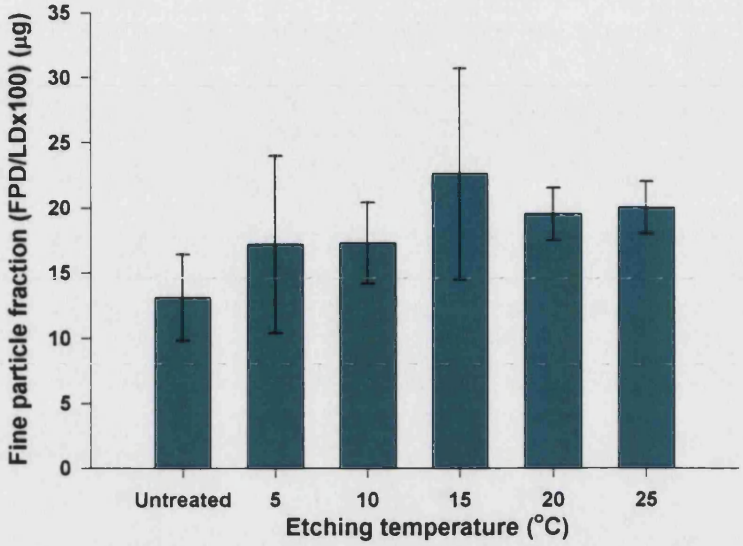

Figure 3.13. Effect of surface etching temperature on the deposition profile of salbutamol sulphate in terms of % fine particle fraction.

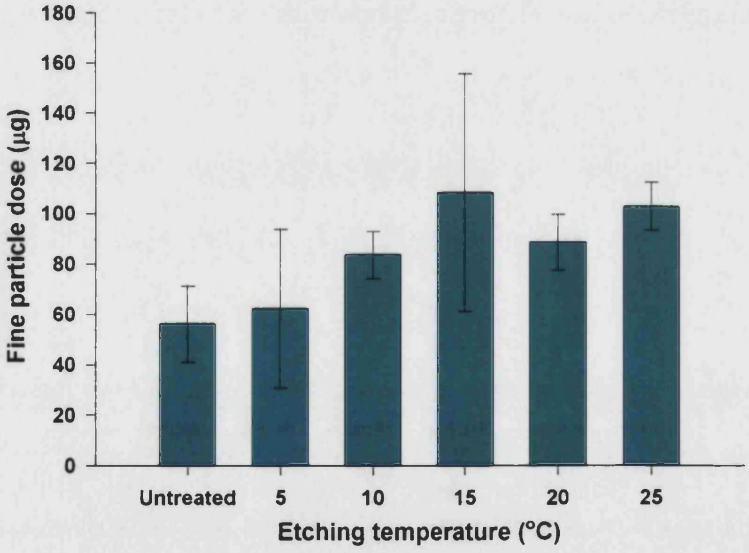

Figure 3.14. Effect of surface etching temperature on the deposition profile of salbutamol sulphate in terms of fine particle dose.

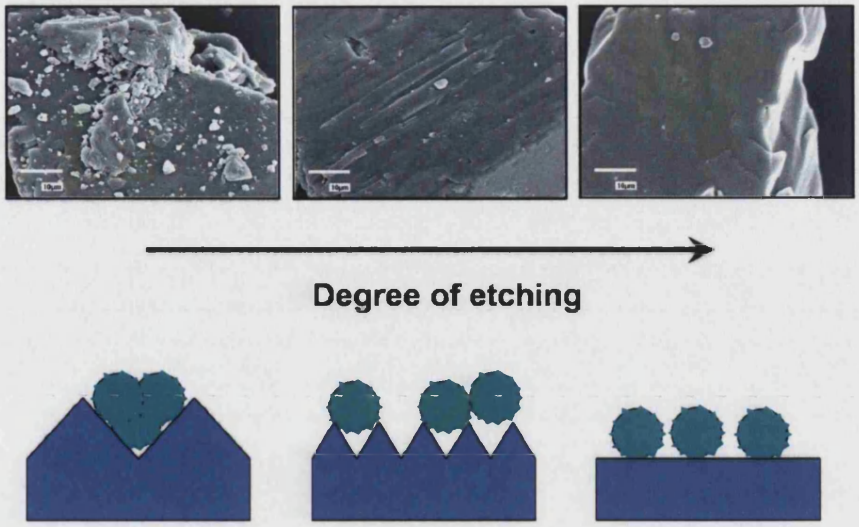

Figure 3.15. Schematic diagram of proposed drug-carrier particles interactions as a function of increased degree of surface etching.

As previously stated, there are conflicting reports about the inter-relationships between lactose based carriers, micronised drugs and apparent formulation efficiency. It was reported that increasing the surface 'smoothness' of lactose improved the performance of inhalation formulations (Ganderton and Kassem, 1992). Presently, it is generally accepted that particle size, surface roughness and the level of fines are of significant importance. Lactose carriers have been modified using a variety of processes to produce excipient particles which exhibit improved aerosolisation properties. Such processes include the use of crystallisation techniques to produce 'smooth' crystals with well defined size descriptors (Zeng *et al.*, 2000a; Larhrib *et al.*, 2003b), complete recrystallisation of carrier particles to increase surface smoothness has also been investigated (Ganderton and Kassem, 1990). Importantly, however, the changes in smoothness of the lactose crystals did not improve the performance compared to the untreated lactose, which was explained in terms of the effect of presence of fines. The same workers reported that increasing the elongation ratio and smoothness results in an increase in aerosolisation of salbutamol sulphate (Zeng *et al.*, 2000a). Additionally it has been reported that the ED, FPD and FPF of terbutaline sulphate increases

with decreasing lactose roughness (Flament *et al.*, 2004). The lactose used in this later study was air-jet sieved to lower the level of fines, reducing the effect of this variable. Lactose has also been 'smoothed' by solution phase treatment of lactose with ternary agents such as magnesium stearate and leucine (Staniforth, 1996a; Young *et al.*, 2002), and dry co-processing with leucine (Lucas *et al.*, 1999). All these methods were reported to result in an improvement in drug aerosolisation. Recently, lactose has been subjected to a wet granulation process using a high shear mixer to reduce surface roughness (Colombo *et al.*, 2000). Another attempt of surface treatment included the wetting of lactose particles with an aqueous-alcohol solution (Iida *et al.*, 2002). This research study also suggests that decreasing the surface roughness of the lactose carrier results in an increase in FPD and FPF without affecting the ED. However, this is not a simple relationship since the etching process also resulted in an increase in the 50th percentile of particle diameter (d_{50}) and a decrease in the level of fines.

However, in this present work, it is interesting to note that the 5% mass etched lactose sample exhibited a dramatic increase in d_{50} and reduction in fines but no increase in the aerosolisation of salbutamol sulphate. This is in agreement with a 'smoothing' study which also included an aqueous method which as well as smoothing the lactose also reduced the level of fines (Young *et al.*, 2002). However, as observed for the 5% mass etched lactose sample in this study, this did not result in any improvement in lactose functionality. This would, in part, agree with the proposal that the presence of a certain level of fines improves the functionality of lactose (Staniforth, 1996a; Lucas *et al.*, 1998a; Zeng *et al.*, 1998; Zeng *et al.*, 1999; Louey *et al.*, 2003; Islam *et al.*, 2004a) and agrees with the suggestion that removing fines and reducing the surface roughness of sieved lactose increases the performance of a DPI formulation (Flament *et al.*, 2004). There are limitations however, for example, even though crystallisation processes can produce lactose with improved aerosolisation, increases in the crystal aspect ratio can be detrimental to formulation characteristics such as content uniformity and flow properties (Larhrib *et al.*, 2003a).

Quantitative comparisons with the literature are difficult due to the variations in the processing and source of the lactose employed and the methodologies utilised in particle size analysis. For example, the classification of a lactose sample based on sieving can be misleading since adhesion of fines to larger particles will always occur due to simple physics. This also confirms that the apparent particle size distribution of lactose may differ depending on the method of determination (Larhrib *et al.*, 1999). These have obvious formulation implications. This was exemplified in a study carried out to determine the relationship between sonication time and the apparent particle size of untreated sieved lactose. Clear differences between the particle size distributions of the untreated lactose were observed. In general, sonication resulted in an increase in smaller particle fractions, most likely due to the separation of fines from the larger particulates. As expected, this was not observed for the surface etched lactose samples, correlating with the previously described SEM images. Minor differences in the particle size distribution were observed with increased dissolution etching temperature but the distribution profiles were very similar.

These observations show that a sieved lactose sample should be considered as a pseudo-ordered or interactive mix rather than a material defined by some experimentally determined size descriptor and that the particle sizing method should be developed to maximise the information from any particle size descriptors obtained. Clearly, the production of a modified lactose carrier, such as the temperature controlled surface etched lactose developed in this research study, may indeed offer improved aerosolisation of drug and formulation characteristics. Furthermore, facilitating a better understanding of particle size and functionality relationships by reducing the effect of the methodology of particle size determination.

3.5 Conclusions

Overall it appears that there is a relationship, albeit a complex one, between lactose nominal particle size, particle size distribution (including fines) and surface topography. This present study suggests that the surface treatment of lactose *via* the controlled etching process with can control the particle surface properties and the removal of fines to produce carriers which increase the aerosolisation of salbutamol sulphate from a single dose inhaler. Additionally, such carriers may exhibit particle size characteristics which are less dependent on sizing methodology.

Chapter 4

The influence of drug to carrier ratio on the delivery performance of low dose inhalation formulations

4.1 Introduction

Over recent years, extensive research studies have been conducted in the exploration of the 'ideal' formulation strategy upon which a satisfactory dry powder inhalation system can be routinely formulated (Newman *et al.*, 1991; Staniforth, 1995; Labiris and Dolovich, 2003). The perception of such a strategy involves improving the flow properties and the aerosolisation characteristics of the respirable-sized therapeutic ingredient. As previously discussed, the requirement of a specific aerodynamic particle diameter for respiratory delivery (0.5-8 μm) (Ganderton and Kassem, 1992), involves subjecting the therapeutic material to high shear secondary processing, such as micronisation and milling (Vidgren *et al.*, 1987).

The processing of the particles gives rise to highly cohesive (towards like particles) and adhesive (towards unlike particles) interactions. In an attempt to alleviate the undesirable shortcomings of having pronounced cohesive/adhesive properties, the micron-sized drug particles are commonly blended with coarse inert carrier particles. The binary interactive system created is termed as an 'ordered mixture' (Hersey, 1975). An ordered mixture, as described by Hersey, involves an interaction between substance

A and substance B that are most likely of unequal size, weight and ratio, yet still contemplating a significant and acceptable degree of powder mixture homogeneity. In fact they are considered to be more homogenous than random mixtures. Hersey also suggested that a definite number of fine particles of substance A may adhere to a single large particle of substance B for any given binary system (Hersey, 1975).

It is well understood that a number of factors influence the interparticulate forces which dominate the interaction between substance A and substance B (Hersey, 1975; Staniforth, 1987). This ultimately influences the detachment of the fine particles of substance A from the surfaces of substance B. These factors include the physicochemical properties of both interacting substances and the environmental conditions under which the final mix was prepared, stored and used (Staniforth *et al.*, 1981; Staniforth *et al.*, 1982; Byron *et al.*, 1996; French *et al.*, 1996; Price *et al.*, 2002; Voss and Finlay, 2002).

Of all the physicochemical parameters governing the delivery performance of a dry powder inhaler system, the surface rugosity of an excipient particle is undoubtedly one of the key determinants in achieving effective pulmonary delivery. The industrial manufacturing of pharmaceutical excipients, such as lactose, is usually carried out through implementing various procedures which have long been used for the relatively large scale extraction of such excipients from their natural resources. That being so, the physical properties and stability of excipient particles are being constantly jeopardised. Such instability resides in obtaining particles that show evidence of irregularities in surface roughness parameters (peaks and troughs). Furthermore, specific regions with interchangeable (both) crystalline and amorphous domains are most likely to be introduced during processing. This may lead to considerable variations in surface free energy and electrical charge among the thermodynamically unstable sites upon their formation (Muster and Prestige, 2002; Begat *et al.*, 2003).

A combination of the factors discussed above contributed to the postulation of the 'active site' theory (Hersey, 1975). The 'active site' theory proposed

that the surface of a large carrier particle consisted of distinct regions so called 'active' high adhesion sites and 'passive' low adhesion sites. Further development described the presence of these sites through the prediction of a surface energy map (Staniforth, 1996b). The surface energy map ideally forecasted 'active' sites as the sites that possess high surface energy, and 'passive' sites as the sites that possess low surface energy. In a similar manner, sites that would yield equivalent adhesional forces between the drug and the carrier interacting surfaces were described as iso-energetic adhesion sites (Staniforth, 1996b).

Under these conditions, it is reasonable to assume that the active high energy sites existing on the surface of the carrier particle would be the ones preferentially filled with finer particles. The finer particles may either be drug particles, intrinsic/extrinsic fine carrier particles, and or fine particles of a ternary component.

The occupation, and thus the complete saturation of these active sites, would therefore be significantly dependant on the ratio of fine particulates which are incorporated into a binary system. The binary system will be one of two possible systems: (*a*). A system of a low ratio of fine particulates, where a considerable amount of fines are strongly adhered to the active sites, due to the sub-filling effect of such sites. (*b*). A system of a high ratio of fine particulates, leading to complete saturation of active sites due to the filling effect of these highly energetic sites. Thus, any remaining fines will be more weakly adhered to passive low energy sites of the excipient surface.

It is clearly evident that the presence of active sites would have significant implications to the drug delivery efficiency from a dry powder inhaler system. However, one can assume that the detrimental influence of active sites would be much more exacerbated if the DPI system was designed to deliver low doses of drugs having high therapeutic potency (e.g. formoterol fumarate which has a formulated dose of 6-12 µg). Since the amount of drug incorporated in such a system is expected to be way below the saturation limit of the active sites, this may result in strong drug-excipient adhesion

behaviour, deviations from target doses and label claim may be a significant issue. On the other hand, the possible influence of active sites may not be of an issue for the majority of marketed DPI formulations which are aimed for the delivery of relatively high doses (e.g. Ventolin Accuhaler®, Ventodisks®, Pulvinal®, and Cyclohaler® ≥200 µg). This is due to the fact that a significant fraction of the nominal dose is actually utilised to fill the active sites, thus facilitating the detachment of the remaining part of the dose under any sufficiently given inspiratory effort.

Several approaches have been employed to overcome the unpredictable and disadvantageous effects of active sites. These approaches, for instance, include the use of increased fine particle content in order to enhance the filling of the potential active sites present on the surface (Lucas *et al.*, 1998b; Zeng *et al.*, 1998; Zeng *et al.*, 1999; Louey and Stewart, 2002; Louey *et al.*, 2003). Another approach developed to tackle this issue involved modifying surface texture and rugosity (Colombo *et al.*, 2000; Iida *et al.*, 2002; Islam *et al.*, 2004; Flament *et al.*, 2004). In addition, previous studies have also investigated modifying the morphology through crystal engineering (Kassem and Ganderton, 1990; Kawashima *et al.*, 1998a; Zeng *et al.*, 2000a; Larhrib *et al.*, 2003b). Moreover, another alternative mean which was also carried out was the use of a ternary force control agent (Staniforth *et al.*, 1982; Lucas *et al.*, 1998a; Lucas *et al.*, 1999; Clarke *et al.*, 2001; Young *et al.*, 2002). In light of these findings there still remains a more comprehensive interpretation of the imperative role of active sites particularly for low dose drug delivery.

The primary aim of this part of the study is concerned with examining the influence of drug to carrier ratio on the aerosolisation performance of a model DPI system and the influence of the physical properties of lactose carrier. In order to achieve a more valuable insight into the rather ambiguous role of active sites, the drug to carrier ratio study was performed on dry powder formulations which exhibited different lactose carriers. The lactose carriers employed were untreated commercial grade α-lactose monohydrate, untreated air jet sieved lactose and surface etched lactose, which underwent

treatment *via* the novel temperature controlled surface etching process, described in Chapter 3.

4.2 Materials

α-Lactose monohydrate (Lactochem® crystals) was supplied by Borculo Whey (Chester, UK). The bulk powder was vibrated through a nest of sieves to obtain a 63-90 µm sieve fraction, which was used throughout the study. Micronised salbutamol sulphate was supplied by Aventis Pharma (Cheshire, UK). Water used was purified by reverse osmosis (MilliQ, Molsheim, France). All solvents used throughout the study were supplied by BDH (Poole, Dorset, UK) and were of at least of analytical grade.

4.3 Methods

General methods of each technique or apparatus used in this study are described in detail in Chapter 2.

4.3.1 Preparation of powder blends

Powder formulations containing different dose levels of salbutamol sulphate were prepared by varying the ratio of salbutamol sulphate to lactose carrier. Eight blends with ratios ranging from approximately 1:100 to 1:5000 w/w were prepared with as received commercial grade α-lactose monohydrate. Seven blends with the same range of drug/lactose ratios were prepared incorporating the 5% surface etched α-lactose monohydrate. Finally, six blends again with the same range of drug/lactose ratios were also prepared with air jet sieved α-lactose monohydrate. The air jet sieved lactose was obtained *via* subjecting the commercial grade lactose to a further air jet sieving process, to remove intrinsic fines. Each formulation was designed

such that each 50 mg of the powder blend contained the desired dose. The volume of the powder in the mixing cell was kept constant to reduce the effect of friction and to maintain reproducibility.

Salbutamol sulphate was blended with the lactose geometrically. Briefly, an amount of lactose, equivalent to about twice the total mass of salbutamol sulphate was used to 'sandwich' the drug between two layers of the lactose powder. This was mixed for one minute using a Whirlymixer (Fisons Scientific Equipment, Loughborough,UK). Lactose was then added in geometric quantities, mixing with a Whirlymixer for one minute upon each addition. The final blend was then placed in a Turbula (Bachofen, Basel, Switzerand) and blended at 46 rev.min^{-1} for a further 30 minutes.

The drug doses formulated with untreated commercial grade lactose were approximately 11 µg, 30 µg, 40 µg, 90 µg, 135 µg, 190 µg, 350 µg and 450 µg. The drug doses formulated with surface etched lactose were approximately 32.5 µg, 43.5 µg, 63.5 µg, 117 µg, 180 µg, 345 µg and 437 µg. Finally, the dose levels formulated with untreated air jet sieved lactose were approximately 30 µg, 55 µg, 91µg, 196 µg, 338 µg and 458 µg.

Each blend was stored in tightly sealed containers with a saturated solution of potassium carbonate (43% RH) for a minimum of 48 hours prior to analysis.

4.3.2 Drug content determination

Quantification of salbutamol sulphate content uniformity and *in vitro* deposition measurements were carried out by high performance liquid chromatography (HPLC). The HPLC system consisted of an AS950 intelligent sampler, PU-980 intelligent HPLC pump, 975 UV/VIS detector (all Jasco, Japan) and Spherisorb 15 cm, 5 µm ODS1 column.

The mobile phase used throughout the investigation was methanol/water (60:40) and acetic acid 0.1 % v/v. Settings were as follows; detection wavelength 276 nm; flow rate 1.25 $ml.min^{-1}$; pressure approximately 400 $Kg.m^2$; injection volume 100 µl; analysis time 4 min; approximate retention time 2 min.

Linearity was confirmed between 0.1 and 10 $\mu g.ml^{-1}$ ($R^2 = 0.999$). Lactose was shown not to interfere with the salbutamol sulphate response. Sample injections were performed in duplicate using a bracket standard method containing standards prepared from separate stock solutions.

4.3.3 In vitro aerosolisation studies

The influence of drug-lactose ratio on the aerosolisation performance was investigated using the twin stage impinger (TSI) (Copley Scientific Ltd., Nottingham, UK). The methodology used followed that of the British Pharmacopoeia. Briefly the TSI contained 7ml of mobile phase in stage one and 30 ml mobile phase in stage two, which at 60 $L.min^{-1}$ produced a cut off mass median aerodynamic diameter of 6.4 µm between the two stages. The flow through the TSI apparatus was controlled using a GAST rotary vein pump and solenoid valve timer (Copley Scientific Ltd., Nottingham, UK). Flow rate was tested prior to operation using a calibrated flow meter.

A filled (49.90 mg ± 0.20 mg) size 3 gelatine capsule was placed into the dosage chamber of the Cyclohaler®. The mouthpiece was attached to the TSI apparatus and the Cyclohaler® inserted until the end met the inner edge of the rubber mouthpiece. The pump was turned on and allowed to stabilise for 4 seconds before starting the valve-timer for a 5 seconds period. The device was removed carefully; the empty capsule placed into a beaker, and the device was primed and tested with a second capsule. This procedure was repeated until 5 actuations had been conducted.

The TSI apparatus was then dismantled and each stage was washed with mobile phase into volumetric flasks. In addition, the device and capsules were washed into a separate volumetric flask. Appropriate sample dilutions were made prior to testing by HPLC. All values were divided by 5 to approximate a single dose.

The TSI aerosolisation studies were conducted in triplicate for each formulation and were randomised for dose.

4.4 Results and discussion

4.4.1 Particle size analysis

The particle size distributions for both lactose and salbutamol sulphate are shown in Figure 4.1 A and B, respectively. The size distribution for the commercial untreated α-lactose monohydrate lactose sample (volume median diameter) indicated that the majority of particles were between 60 µm and 200 µm in diameter. It is interesting to note, that the upper size was higher than the sieve fraction value (90 µm) and was most likely due to the "tomahawk" geometry of the lactose particles. Furthermore, approximately 6% of the particles exhibited diameters of less than 20 µm, with 2% less than 5 µm. These can be related to the presence of fine lactose particles attached to the large carrier particles which were visible using SEM (See section 3.4.3.2). This demonstrated that simple sieving does not provide sufficient energy to remove adhered lactose fines from larger carrier particles and that lactose fractions produced by sieving consist of a pseudo-ordered mix which has obvious formulation implications. Analysis of the particle size distribution of micronised salbutamol sulphate suggested 90% of the particulates exhibited a diameter less than 9.4 µm and 50% less than 3.4 µm.

The particle size distributions of both surface etched and untreated air jet sieved lactose samples are also presented in Figure 4.2 A and B. It was clearly evident from the particle size distributions, that untreated air jet sieved lactose exhibited a higher level of fine lactose particulates when compared to the surface etched lactose carrier. For surface etched lactose, approximately 8% of particles exhibited diameters of less than 20 µm with around 4% less than 5 µm. Whereas for untreated air jet sieved lactose, approximately 25% of particles exhibited diameters less than 20 µm, with around 10% less than 5 µm. The results obtained were further corroborated with the SEM investigations (Section 4.4.2).

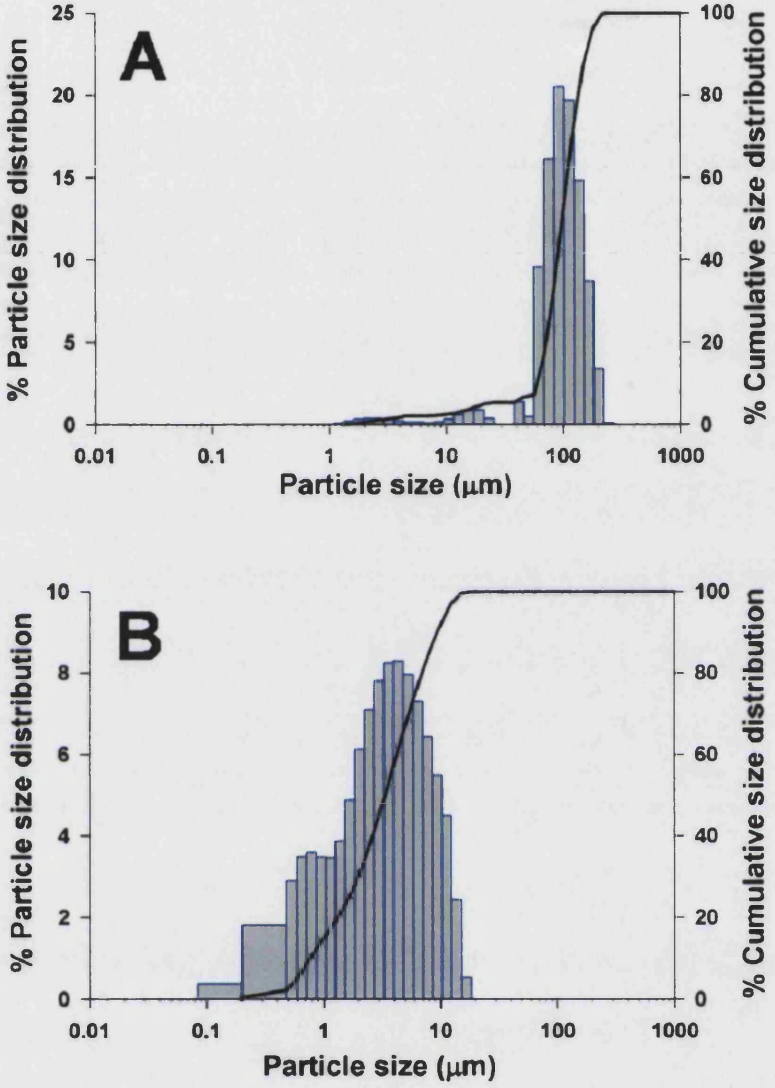

Figure 4.1. Particle size distribution of 63-90 µm sieve fractioned commercial grade α-lactose monohydrate (A) and micronised salbutamol sulphate (B).

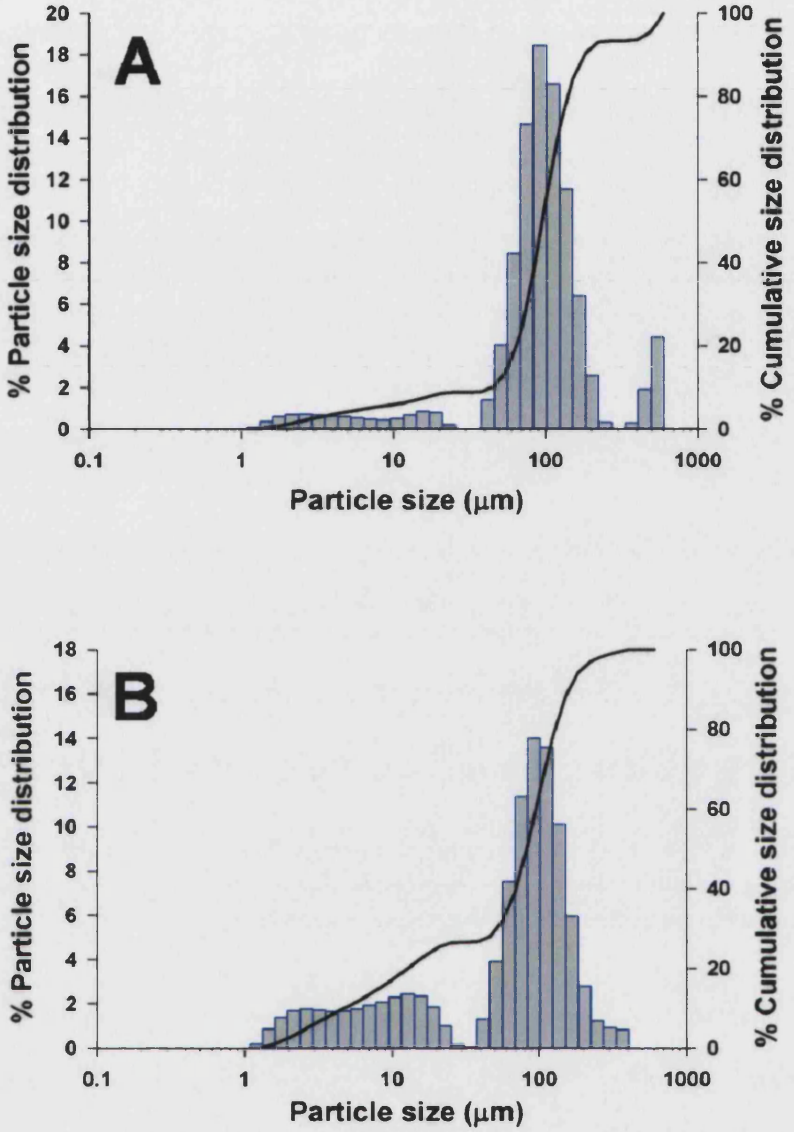

Figure 4.2. Particle size distribution of 63-90 μm sieve fractioned surface etched lactose (A) and untreated air jet sieved lactose (B).

4.4.2 Scanning electron microscopy

Representative scanning electron microscopy (SEM) photomicrographs of unblended and blended surface etched lactose and untreated air jet sieved lactose particles are shown in Figures 4.3 and 4.4, respectively. The

micrograph of the processed lactose particles suggest a distinct variation in the amount of intrinsic fines adhering to the surface of air jet sieved lactose with respect to surface etched lactose, which is clearly evident in Figure 4.3. The particle size analysis and SEM investigations indicate that the air jet sieving process does not adequately remove the desired degree of intrinsic fines. These data suggest that the temperature controlled surface etching process may offer a better processing technique for the accomplishment of a rather uniform particle size distribution with the removal of fine particulate material.

Representative SEM images of 12 µg, 135 µg and 450 µg dose blends of commercial grade α-lactose monohydrate are shown in Figure 4.5 A, B and C, respectively. Representative SEM images of 12.5 µg, 100 µg and 400 µg dose blends of surface etched α-lactose monohydrate are also shown in Figure 4.6 A, B and C, respectively. Clear variation in the degree of fine particulates (< 5 µm) were observed when comparing the blends from the lowest to the highest dose level. In general, a rank-order in fine particulate number matched that of the blend dose. Unformulated lactose SEM images of untreated commercial grade α-lactose monohydrate showed large carrier particles with some smaller adhering lactose particles which is in agreement with the previously described particle size data. A parallel argument can be made when examining the SEM images of both the untreated commercial grade and surface etched lactose blends as represented in Figures 4.5 and 4.6. The three selected dose level blends of untreated commercial grade α-lactose monohydrate demonstrated a relatively higher presence of fine particulates on the larger lactose particles when compared to the almost equivalent dose level blends of the surface etched lactose. Such observations were further emphasised by the SEM photomicrographs of the same 400 µg dose level of surface etched and untreated air jet sieved lactose blends represented in Figure 4.4 A and B. The SEM photomicrograph of the 400 µg untreated air jet sieved lactose blend (Figure 4.4 B) not only displayed a higher level of fine particulates adhering on to the surface, but also, a rougher surface topology of the lactose particle was evident. One can therefore reason that the temperature controlled surface dissolution process

had a significant effect on considerably minimising apparent particle surface irregularities.

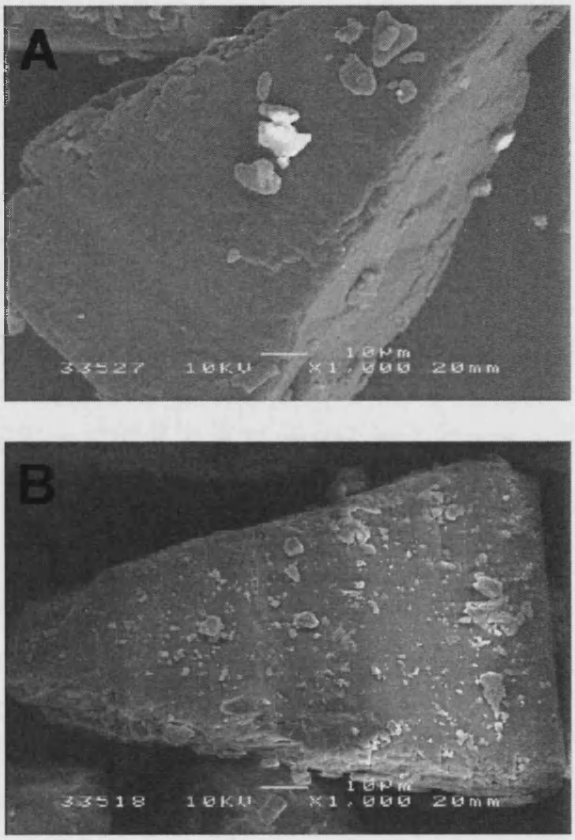

Figure 4.3. Representative scanning electron photomicrographs of surface etched lactose (A) and untreated air jet sieved lactose (B).

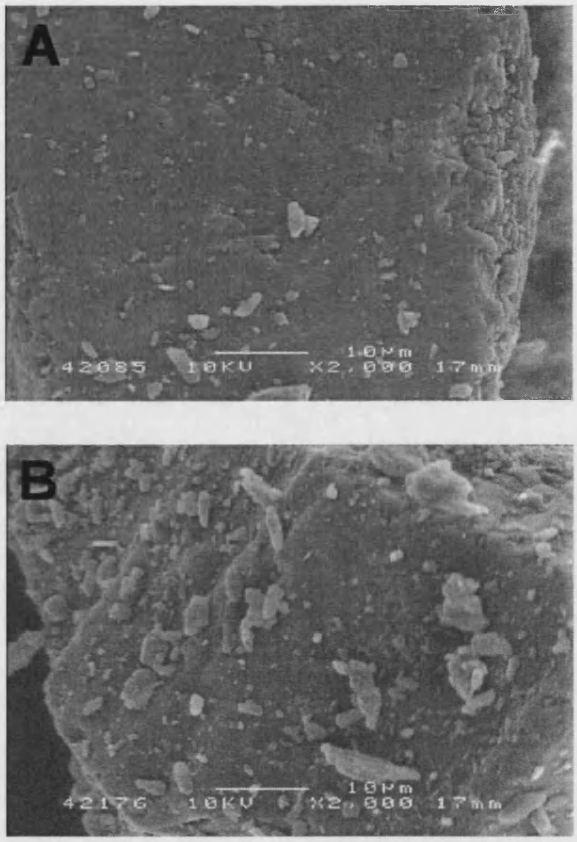

Figure 4.4. Representative scanning electron photomicrographs of 400 μg dose surface etched lactose (A) and untreated air jet sieved lactose (B) blends.

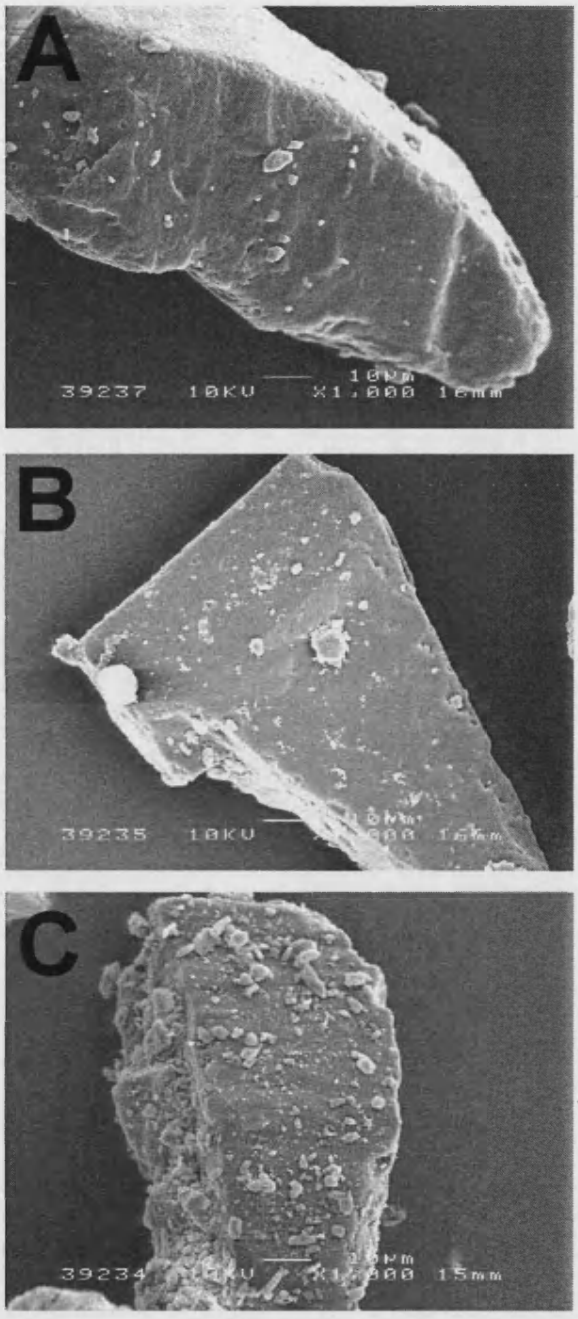

Figure 4.5. Representative scanning electron photomicrographs of 12 µg (A), 135 µg (B) and 450 µg (C) untreated commercial grade α-lactose monohydrate blends.

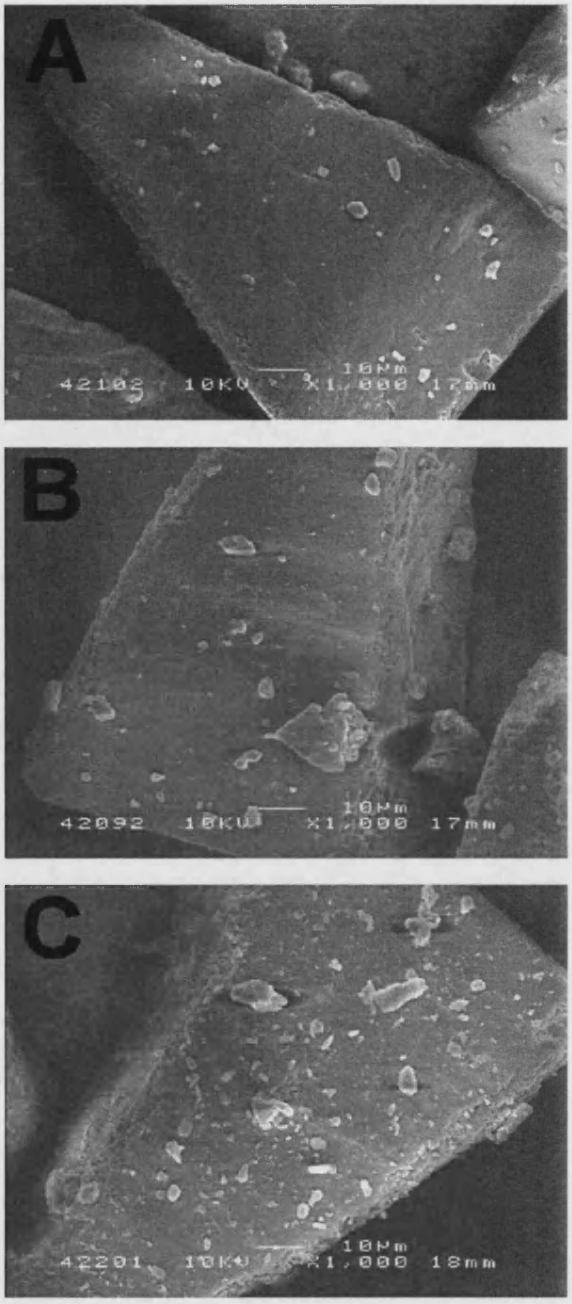

Figure 4.6. Representative scanning electron photomicrographs of 12.5 µg (A), 100 µg (B) and 400 µg (C) surface etched lactose blends.

4.4.3 In vitro aerosolisation studies

Content uniformity (50 mg samples) across each blend indicated a coefficient of variation less than 5% (n=10). The aerosolisation efficiency of salbutamol sulphate from the various lactose carriers was investigated using the twin stage impinger (TSI). Data was processed and represented as follows: loaded dose (LD), which was the drug recovered from the device/capsule, mouthpiece, throat and stages 1 and 2; the emitted dose (ED), was the drug recovered from the mouthpiece adapter, throat and stages 1 and 2; the fine particle dose (FPD), was the drug recovered from stage 2 of the TSI; and the fine particle fraction (FPF), which is the percentage of fine particle dose (FPD) to loaded dose (LD).

The relationship between emitted dose and loaded dose for the untreated commercial grade α-lactose monohydrate formulation is shown in Figure 4.7. The data suggested a linear response (R^2 = 0.998) between loading and emission efficiency, with a nominal device removal efficiency of 89% ± 4% across all doses. A linear relationship was also obtained between the emitted dose and loaded dose for all three lactose carriers under investigation in this part of the study. Comparisons between untreated lactose and surface etched lactose and air jet sieved lactose are shown in Figures 4.8 and 4.9, respectively. This study suggested that the efficiency of the device at removing 50 mg bulk formulation was independent of lactose type or drug/lactose ratio. Moreover, since the percentage drug loss in the device was apparently independent of dose, it is therefore reasonable to assume that such losses are due to drug adhered to retained lactose, since the formulation mass remained relatively constant.

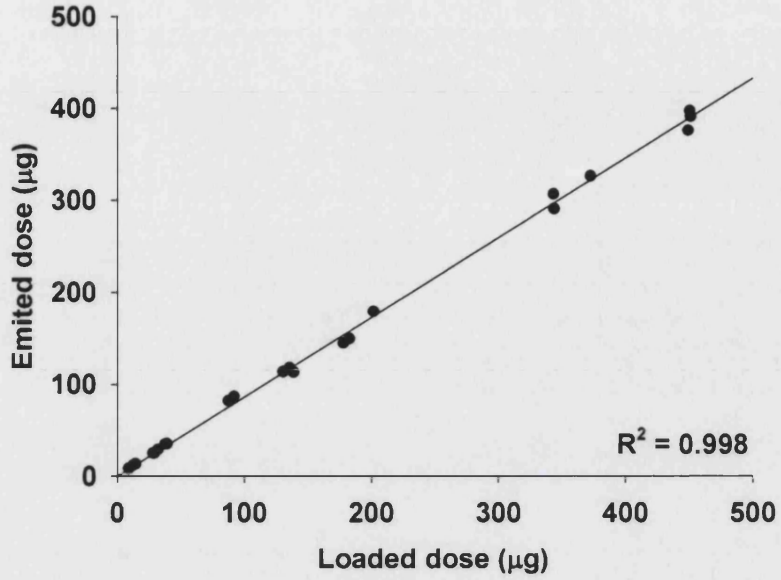

Figure 4.7. Influence of loaded dose on the emitted dose from an untreated commercial grade α-lactose monohydrate formulation.

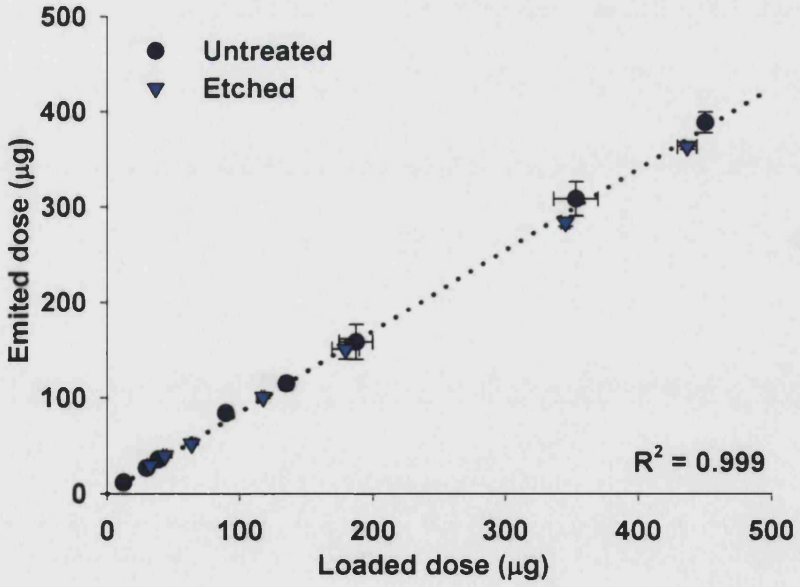

Figure 4.8. Influence of loaded dose on the emitted dose. Comparison between untreated commercial grade and surface etched lactose.

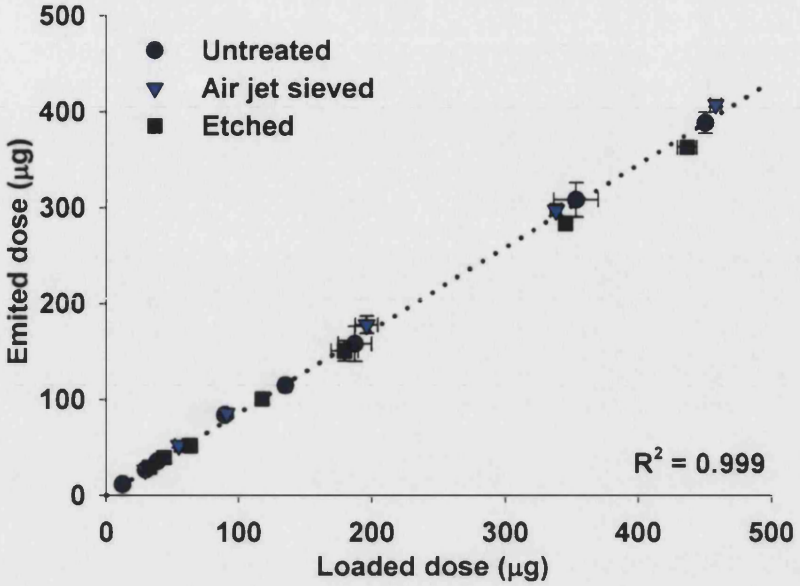

Figure 4.9. Influence of loaded dose on the emitted dose. Comparison between untreated commercial grade, air jet sieved and surface etched lactose.

The relationship between fine particle fraction and loaded dose for the untreated commercial grade α-lactose monohydrate formulation is shown in Figure 4.10 and tabulated in Table 4.1. It can be seen from the graph that the loaded dose has a significant effect on the fine particle performance (ANOVA $p<0.05$). In general, a linear decrease ($R^2 = 0.977$) in fine particle fraction was observed on increasing the dose from 11 µg to 135 µg followed by an increase when the dose was increased from 135 µg to 450 µg.

A rather similar trend was also obtained when examining the relationship between fine particle fraction FPF and loaded dose for the surface etched lactose formulation (Figure 4.11). It was noticeable, however, that the increase in FPF with increasing loading dose took place at a lower dose (Table 4.2). That is to say, a linear decrease ($R^2 = 0.958$) in fine particle fraction was observed on increasing the dose from 32.5 µg to 63.5 µg followed by an increase when the dose was increased from 63.5 µg to 437 µg which is significantly different than the increase that took place at the

dose level of 135 µg for the untreated commercial grade α-lactose monohydrate formulations. Furthermore, a significant difference in the aerosolisation efficiency (FPF) between untreated commercial grade and surface etched lactose was determined at the dose level of approximately 100 µg and above (ANOVA $p<0.05$).

The logical conclusion from such observations would be related to the 'active site' theory. The surface smoothing of lactose particles resulted in a decrease in active sites, and if the quantity of these active sites are reduced, the point at which the linear decrease in FPF with loaded dose may decrease to a lower drug/lactose ratio. Conversely, an increase in the degree of 'active sites' may lead to the requirement for an initial higher loaded dose to achieve a linear relationship between fine particle delivery performance and loaded dose.

No considerable variation in the aerosolisation behaviour of untreated commercial grade and untreated air jet sieved lactose formulations at low and high drug/lactose ratios was determined, as indicated in Figure 4.12 and Table 4.3. Such an observation is expected since the surface of untreated air jet sieved lactose particles exhibited similar morphological properties to commercial grade lactose in terms of surface roughness as observed *via* the SEM photomicrographs. It is these variations in surface features that may be the source of the high energy active sites that are initially filled with strongly adhering drug particles at low doses. This would result in the need for a critical concentration of drug to saturate active sites. It is beyond this critical concentration that a further increase in the drug load (drug/lactose monohydrate ratio) may result in a linear increase in both FPF and FPD with loaded dose.

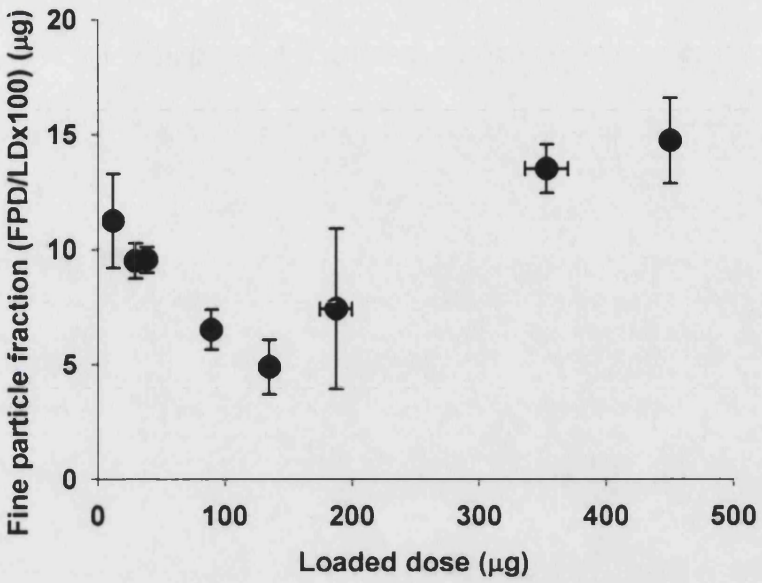

Figure 4.10. Influence of loaded dose on fine particle fraction from an untreated commercial grade α-lactose monohydrate formulation.

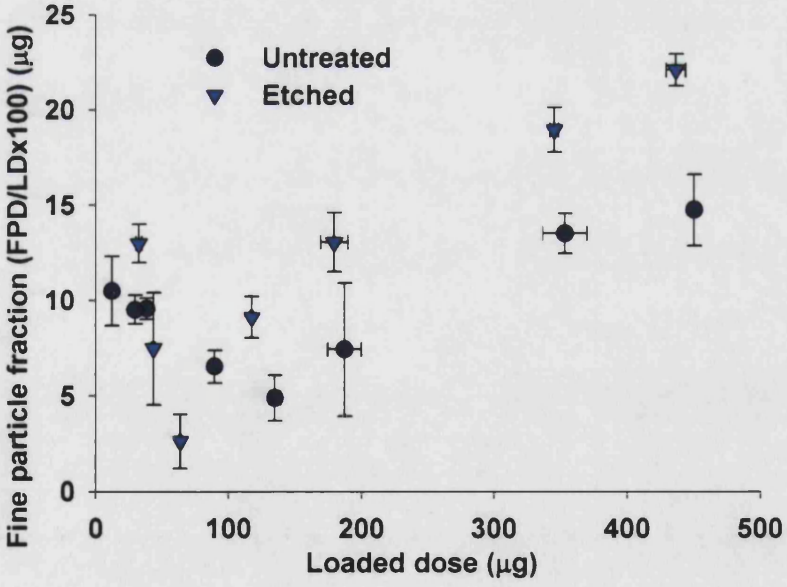

Figure 4.11. Influence of loaded dose on the fine particle fraction. Comparison between untreated commercial grade and surface etched lactose.

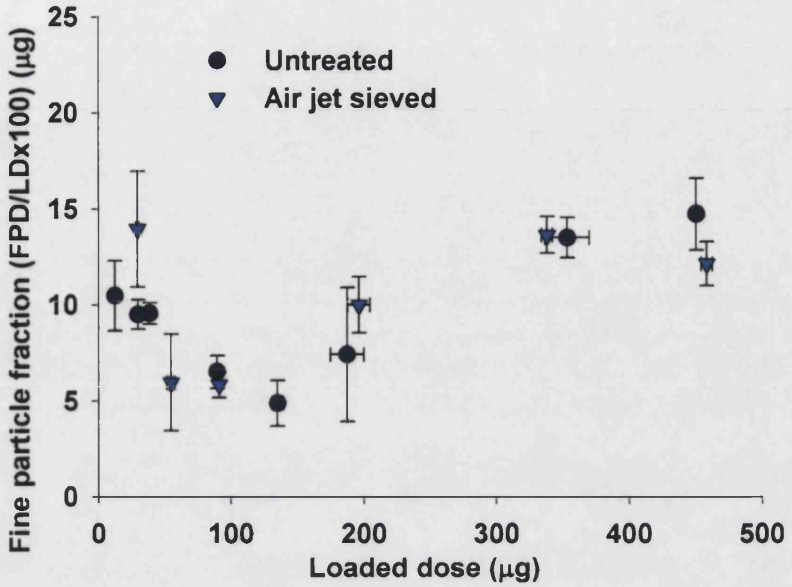

Figure 4.12. Influence of loaded dose on the fine particle fraction. Comparison between untreated commercial grade and untreated air jet sieved lactose.

The relationship between fine particle dose and loaded dose of untreated commercial grade, surface etched and air jet sieved lactose formulations is shown in Figures 4.13 and 4.14. The data suggested that for the untreated commercial grade lactose formulations, the dose level only had a statistically significant effect on the FPD measurements once it was higher than 135 µg (ANOVA, Fishers's pairwise $p<0.05$). Analysis of the FPD with loaded dose for the surface etched lactose formulations (Figure 4.14) indicated no significant differences in performance between loaded dose for concentrations up to 63.5 µg. Such observations suggest that since both samples were 63-90 µm sieve fractioned lactose that exhibited similar particle size distributions, and there was no evidence of drug agglomeration, the smoothing of lactose monohydrate resulted in a decrease in 'active sites'. Thus, may provide the potential for improvement of drug aerosolisation at lower doses.

These observations above can be corroborated when investigating the

FPF (Figure 4.11). As previously discussed, when studying untreated commercial grade formulations, a linear decrease in FPF was observed as the potential 'active sites' were filled resulting in no significant difference in FPD, prior to an increase. With the smoother surface dissolved lactose monohydrate (potentially containing less active sites), the slope and point of minimum linear decrease was reduced (minimum FPF at drug load = 63.5 µg R^2 0.958 for the etched lactose, compared to a minimum of FPF at drug load = 135 µg R^2 0.977 for the commercial grade lactose).

It is significant to note, however, that the relationship between the loaded dose and FPF across the dose range 63.5 – 437 µg for etched lactose and 135 – 450 µg for commercial grade lactose was not perfectly linear (Figure 4.11). This is to be expected however, since such a system would still contain a certain distribution of active sites below the critical adhesion limit and therefore potentially result in a non-uniform performance (i.e. the probability of drug-particle removal will be dependent on the filling of differing energy sites). Furthermore, the inherent particle size distribution of the micronised drug would set an upper fine particle fraction limit.

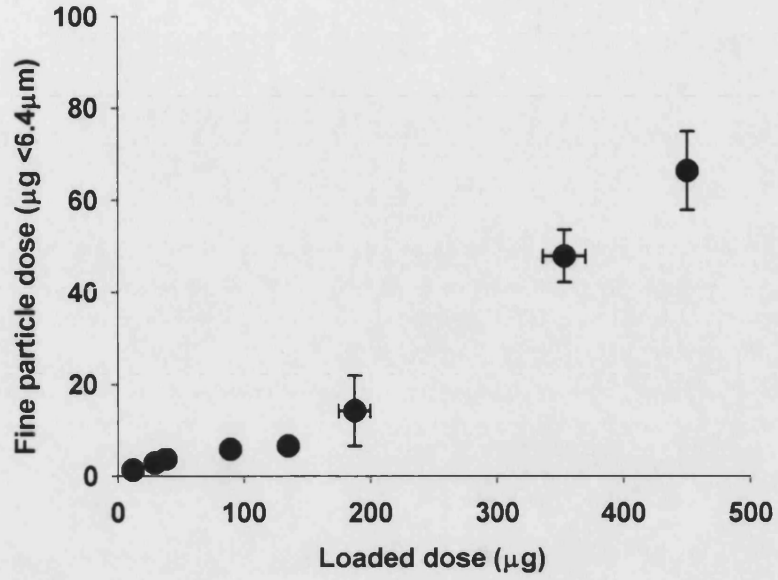

Figure 4.13. Influence of loaded dose on fine particle dose from an untreated α-lactose monohydrate formulation

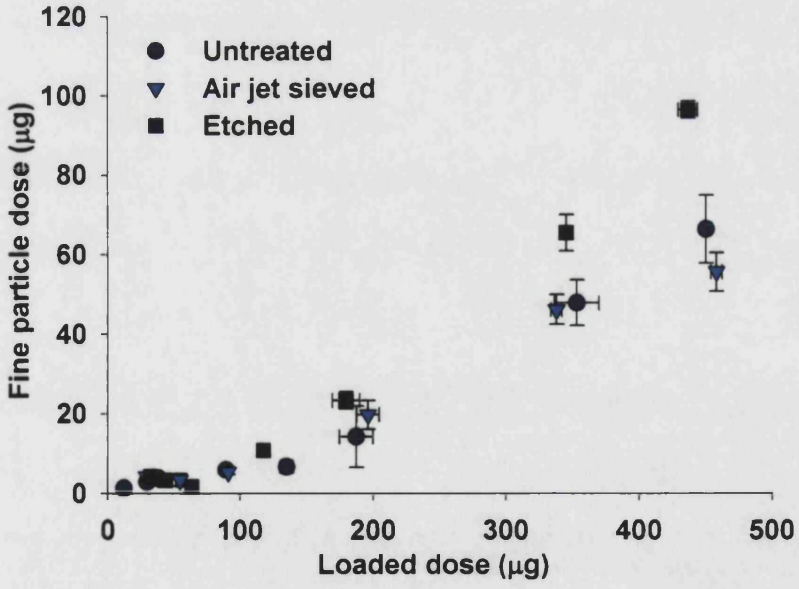

Figure 4.14. Influence of loaded dose on fine particle dose. Comparison between untreated commercial grade, untreated air jet sieved and surface etched lactose.

	Commercial grade lactose		
Loaded dose (µg)	ED (µg)	FPD (µg)	FPF (%)
12.1 ± 2.4	11.3 ± 2.3	1.2 ± 0.1	10.5 ± 1.8
29.7 ± 2.1	26.5 ± 2.3	2.8 ± 0.3	9.5 ± 0.8
38.4 ± 0.8	35.2 ± 0.2	3.7 ± 0.3	9.6 ± 0.6
89.4 ± 2.1	83.9 ± 2.5	5.8 ± 0.7	6.5 ± 0.9
134.9 ± 4.1	114.9 ± 2.4	6.6 ± 1.5	4.9 ± 1.2
187.4 ± 12.5	158.2 ± 18.4	14.2 ± 7.7	7.4 ± 3.5
353.2 ± 16.6	308.1 ± 17.8	47.8 ± 5.7	13.5 ± 1.1
450.4 ± 1.0	388.1 ± 10.9	66.4 ± 8.5	14.7 ± 1.9

Table 4.1. Influence of loaded dose on the aerosolisation parameters of commercial grade α-lactose monohydrate formulations.

	Surface etched lactose		
Loaded dose (µg)	ED (µg)	FPD (µg)	FPF (%)
32.5 ± 2.2	29.5 ± 2.4	4.2 ± 0.6	13.0 ± 1.0
43.5 ± 1.3	39.3 ± 2.1	3.3 ± 1.4	7.5 ± 2.9
63.5 ± 0.8	52.1 ± 1.2	1.7 ± 0.3	2.6 ± 1.4
117.3 ± 1.1	100.9 ± 1.4	10.7 ± 1.3	9.1 ± 1.1
179.4 ± 10.3	150.7 ± 10.6	23.3 ± 2.2	13.0 ± 1.6
345.3 ± 3.1	283.8 ± 4.6	65.4 ± 4.6	18.9 ± 1.2
436.9 ± 7.2	363.0 ± 2.9	96.4 ± 2.1	22.1 ± 0.8

Table 4.2. Influence of loaded dose on the aerosolisation parameters of surface etched α-lactose monohydrate formulations.

	Air jet sieved lactose		
Loaded dose (µg)	ED (µg)	FPD (µg)	FPF (%)
29.4 ± 1.4	26.7 ± 1.4	4.1 ± 0.9	13.9 ± 3.0
54.7 ± 2.9	52.3 ± 2.5	3.3 ± 1.6	6.0 ± 2.5
90.8 ± 0.6	85.8 ± 1.3	5.3 ± 0.6	5.8 ± 0.7
196.2 ± 8.5	178.1 ± 9.0	19.7 ± 3.6	10.0 ± 1.5
338.1 ± 4.1	297.4 ± 5.9	46.1 ± 3.8	13.6 ± 0.9
458.4 ± 4.2	407.3 ± 2.3	55.6 ± 4.9	12.1 ± 1.1

Table 4.3. Influence of loaded dose on the aerosolisation parameters of air jet sieved α-lactose monohydrate formulations.

As previously discussed, there are many possible topographical features that a drug particle may encounter on a lactose surface, such features exhibit a considerable degree of variations in morphology and surface free energy as schematically represented in Figure 4.15 A. As a consequence of a combination of increased contact area, high surface free energy and simple geometric constraints, it is envisaged that sites with high energy (area 1 in Figure 4.15 A) on the carrier surface would be preferentially occupied compared to sites with low energy (area 2 in Figure 4.15 A).

A possible example of this is shown in Figure 4.15 B, where micron sized particulates have accumulated in a recess in the surface of a large lactose carrier particle.

Furthermore, it is suggested that the active sites present on the surface of the carrier will have a specific energy distribution (Figure 4.15 C) with a critical, average adhesion point below which particles, drug or lactose, could be removed. This concept correlates with previous studies, which have suggested that surface roughness and carrier material directly influence aerosolisation of drug from lactose carriers.

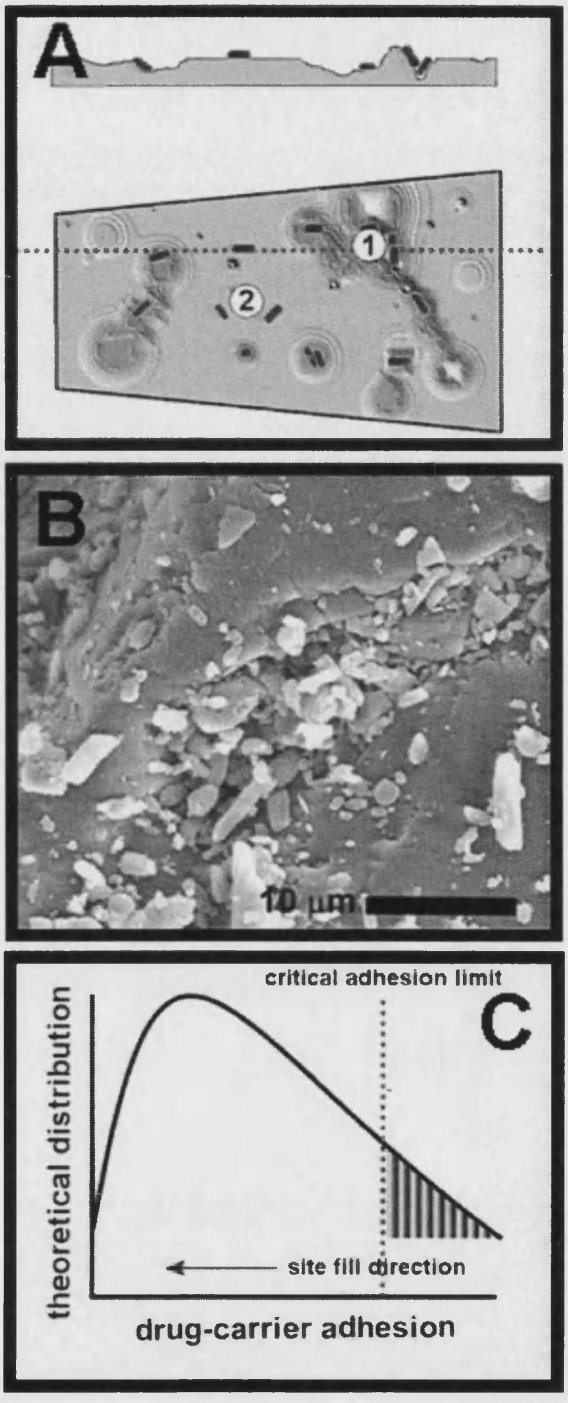

Figure 4.15. Schematic diagram of regions on a carrier surface (A) containing potential high energy (1) and low energy (2) 'active' sites. SEM of a crevice on a lactose carrier surface containing many micron sized particulates (B). Theoretical distribution and process of active site filling (C).

Another point to consider is the potential for the formation of drug or drug-lactose fines agglomerates. Previous studies have reported that the presence of fines increase the fine particle fraction through the formation of agglomerates or multiplets (Lucas *et al.*, 1998a). However, recent studies have suggested that the agglomeration or individual drug-carrier formation of a blend will be related to the balance of adhesion and cohesion in the system (Begat *et al.*, 2004). It was suggested that for a salbutamol sulphate-lactose system, adhesion would dominate, thus reducing the potential for agglomeration (Begat *et al.*, 2004). Such observations correlate well with the SEM images of the blends in this study, which suggested many of the micronised particulates in the salbutamol lactose system to be distributed as discrete entities. However, it is important to remember that the formulation mechanism will be dependent on the drug and carrier properties.

Recent studies by Louey *et al.* (2003) suggested that increasing the presence of lactose fines result in an agglomerate based system. This is likely, since the potential free carrier space would be reduced. Furthermore, it is envisaged that the fine particle fraction would eventually plateau and decrease due to multilayer or aggregate formation and formulation segregation.

Observations across the dose range 32.5 – 437 µg for surface etched lactose and 11 – 450 µg for commercial grade lactose suggested that the disadvantageous influence of so called 'active sites' on the aerosolisation performance has a more dominant effect on formulations aimed at low dose drug delivery and that such influence becomes less disadvantageous upon increasing dose concentration above a critical value. Clearly many variables would influence this relationship and are worth considering for future investigation. These include quantifying the influence of inherent fines and directly relating the influence of modified carrier surfaces to fine particle adhesion.

4.5 Conclusions

Clear variations in the fine particle dose and fine particle fraction were observed as a function of drug load or drug/lactose ratio. The relationship between drug/lactose ratio and aerosolisation performance was related to the possibility of 'active sites' present on the lactose carrier surface. From a logical perspective, the reduction of 'active sites' by surface smoothing of lactose particles by controlled dissolution resulted in a significant increase in aerosolisation performance at lower drug concentrations. Although such factors may not significantly affect the majority of current inhalation formulations, which typically contain >100 µg of drug, the performance of lower dose formulations will clearly be more vulnerable to the influence of 'active sites'.

Chapter 5

Investigation into the influence of storage conditions on the delivery performance of dry powder inhalation formulations

5.1 Introduction

It is widely accepted that the attainment of an effective respiratory drug delivery is largely attributed to the interplay of physical and chemical factors which govern particle-particle interactions (Buckton, 1997; Zeng *et al.*, 2001a). The key mechanisms for the delivery of respirable particles from a passive DPI device is the detachment of drug particles from the surface of a carrier particle, dispersion of drug particles into the airflow and finally deposition of drug particles within the respiratory system. Thus, the therapeutic effect of inhaled medicaments is significantly influenced by these mechanisms and the physicochemical factors which govern their behaviour (Ganderton and Kassem, 1992; Byron *et al.*, 1996).

As previously discussed, it is well-understood that the optimum therapeutic benefit of aerosol particles within the respiratory tract is the delivery of aerodynamic diameter particles in the size range 2 - 5 μm (Clark, 1995; Malcolmson and Embleton, 1998). This critical size requirement is associated with efficient delivery within the conducting airways rather than being lost through impaction and/or sedimentation in the upper airways (Prichard,

2001). To achieve the required particle size ranges, the active ingredient is traditionally subjected to vigorous mechanical processing, such as micronisation and milling (Staniforth, 2000). The use of such high energy processing typically leads to production of particles with high surface free energies, due to the introduction of amorphous disorder, and issues relating to electrical charging (Buckton *et al.*, 1988, Krycer and Hersey, 1981; Saleki-Gerhardt *et al.*, 1994; Ward and Schultz, 1995; Ohta and Buckton, 2004).

As a result, the stability of a DPI formulation will be markedly threatened, especially if subjected to an environment displaying pronounced variations in temperatures and/or relative humidity (%RH) levels (Hindle and Makinen, 1996; Price *et al.*, 2002a). For example, thermodynamically unstable amorphous regions have been shown to be highly susceptible to re-crystallisation upon exposure to elevated changes in temperature and/or %RH, leading to significant changes in the physicochemical properties of the bulk powder and the potential for irreversible particle agglomeration (Ahlneck and Zografi, 1990; Hancock and Zografi, 1997).

In view of the fact that inter-particulate interactions at the sub-micron scale are effectively a surface phenomenon, the morphology and crystalline/amorphous characteristics of the surface will indeed exert a significant influence on both the adhesive/cohesive properties of interacting particles and, thus, the physicochemical stability of the formulation upon storage at elevated temperature and %RH conditions.

For respirable sized particles, their interactions are governed by a composite of omni-present van der Waals forces and electrostatic and capillary forces. The magnitude of these physical forces is strongly influenced by particle properties such as particle size, shape, surface energy and surface texture (Podczeck, 1998a). Furthermore, the dynamic influence of the capillary and electrostatic forces is highly dependant on the processing and environmental conditions (Coelho and Harnby, 1978). Electrostatic forces, for example, have been shown to dominate particulate interactions at low RH levels (<30% RH) (Price *et al.*, 2002). Contact electrification arises from the contact

and separation of two different contiguous surfaces exhibiting different energy states, while frictional contact between particles during powder mixing, handling and aerosolisation leads to triboelectrification. This force may be attractive or repulsive (Bailey, 1984; Peart *et al.*, 1996). The influence of contact and tribology induced charging can be significantly minimised in the presence of moist air at reasonable levels of humidity. The condensation and adsorption of water onto interacting surfaces leads to an increase in surface conductivity, thus reducing the potential for the build-up of electric charge (Smeltzer *et al.*, 1982; Rowley and Mackin, 2003). However at high levels of relative humidity (>65% RH), capillary forces may dominate. The adsorption of water onto surfaces causes attractive bonding due to the capillary action of the adsorbed water layers. The sorption of water is highly dependent on the partial water vapour pressure of the surrounding environment, and the hydrophilic/hydrophobic nature of the surface. Significant and increased condensation of water vapour will lead to the formation of liquid bridges *via* capillary action (Coelho and Harnby, 1978; Schubert, 1984), thus leading to a meniscus force and accordingly increased cohesive/adhesive behaviour between particles (Eaves and Jones, 1972).

A number of recent investigations have focussed on the influence of environmental conditions on inter-particulate forces, and subsequently the aerosolisation performance from two variant inhalation systems. The first was concerned with systems exhibiting drug particulates only, therefore concentrating on drug-drug interactions. For example, research has shown that the storage of a variety of micronised salbutamol salts at different temperature/RH levels for various time intervals (3-60 minutes), resulted in a relative decrease in fine particle fraction (Jashnani *et al.*, 1995; Jashnani and Byron, 1996). It is very important to note, however, that the aerosolisation behaviour was not the same for all salts investigated. Some, for example, remained largely unaffected, until subjected to relatively extreme conditions, such as 45°C and 95% RH. Their overall conclusion highlighted that the stearate salt, the most hydrophobic, showed the least sensitivity to elevated temperature and RH levels.

Another study (Young *et al.*, 2003a) investigated the influence of RH on the aerosolisation performance of three micronised drugs, disodium cromoglycate (DSCG), salbutamol sulphate and triamcinolone acetonide (TAA). A decrease in fine particle fraction of DSCG and salbutamol sulphate was observed at variable levels of increased RH, suggesting that the particulate interactions were mainly governed by capillary forces at high humidity. Surprisingly, however, fine particle fraction of TAA significantly increased as the humidity increased over the range of 15 to 75% RH, suggesting that triboelectric forces were governing the particulate interactions at low to high humidities. Their work was further advocated through the use of the atomic force microscope for direct quantification of the separation energies between drug particulates as a function of increased RH (Young *et al.*, 2003b).

For carrier based formulations, environmental conditions will unquestionably exert a relatively profound influence on particulate interactions and, thus, aerolisation performance. This is due to the large number of interactions: drug-drug, drug-carrier, or even carrier-carrier interactions which will be directly influenced by variations in temperature and/or relative humidity. A recent study, has investigated *in vitro* inhalation properties of a model drug from an inhalation system with surface modified lactose, compared to another formulation of unmodified lactose, both stored at elevated RH levels (Iida *et al.*, 2004a). The modified lactose was magnesium stearate processed lactose. The study indicated that, when tested following storage, the modified lactose formulation was not affected as much as the unmodified lactose formulation. It was suggested that the hydrophobic nature of magnesium stearate, thus its low susceptibility to moisture uptake, prevented the formation of strong capillary bridges between drug and carrier particles, leading to significantly weaker adhesive interactions. Braun *et al.* have undertaken a more detailed investigation into the influence of storage humidity on the deposition behaviour of a model drug from carrier based formulations with lactose excipient particles of differing particle size ranges and drug/carrier ratios (Braun *et al.*, 1996). The study showed that upon storing at 55% RH, a decrease in fine particle fraction was observed for all

formulations in comparision to that obtained at 33% RH. The highest fine particle fraction was obtained from formulations containing one to one drug/carrier ratio with fine carrier particles (d_{50} = 15.6 µm) at 41% RH. It was evident from such an investigation that the inhalation efficiency could be to some extent maintained through the appropriate selection of the excipient in terms of its particle size distribution and its proportion within the formulation. All of which however, needed to coincide with the optimum environmental condition at which the formulation was stored.

In a similar approach, Harjunen and co-workers studied the effect of carrier type, drug/carrier ratio and one month storage at 40°C, 75% RH on the *in vitro* pulmonary deposition of two model drugs. The drugs used in the study were budesonide (hydrophobic) and salbutamol sulphate (hydrophilic). It was observed that following one month storage, the fine particle fraction of salbutamol sulphate decreased and its behaviour was independent of the carrier type and drug/carrier ratio. However, the fine particle fraction of budesonide was found to increase when increasing drug to carrier ratio, and its behaviour was dependent on the carrier type (Harjunen *et al.*, 2003). The increase in fine particle fraction performance of budesonide particles upon storage is thought to be due to the reduction of surface electrostatic properties (Harjunen *et al.*, 2003). Furthermore, the increase in fine particle fraction of budesonide with increasing its dose, was suggested to be related to the presence of 'active sites'. When the drug concentration was low, drug particles would initially occupy high energy adhesion sites, thus lowering the chance of drug particles being detached from the surface. Whereas at higher doses, the probability of drug particles adhering to low energy adhesion sites would increase, hence facilitating more efficient particle detachment (Harjunen *et al.*, 2003). It is significantly important to note, however, that such increase was in part correlated with the increase in drug to carrier ratio. Such results, for instance, seem to be contradictory at first sight to results obtained by a study carried out by Price *et al.* The outcome of this study indicated that capillary interactions were dominating the adhesion forces between budesonide and lactose particles at levels from 15 to 60% RH. It was suggested that such observations were possibly due to the fact that the

unstable hydrophobic nature of the surface of budesonide particles which lack the presence of a saturated tightly bound water layer, rendered it more prone to the adsorption of thin water films which could progressively lead to the formation of a stable meniscus bridge (Price *et al.*, 2002a). Further interesting evidence of the influence of the configuration of an inhaler formulation on its physicochemical stability was also significantly correlated with the findings of such investigations. A study carried out by Mueller-Walz and Keller (Mueller-Walz and Keller, patent WO0028979) examined the impact of the addition of magnesium stearate and fine particle lactose on retaining short and long term stability of lactose based dry powder inhalation systems. In general, their study concluded that the presence of magnesium stearate improved the stability of formulations by exhibiting relatively minimal decrease in FPD and FPF following storage. On the other hand, the addition of fine particle lactose, although showing an initial increase in FPD and FPF when tested before storage, showed a dramatic decrease when tested after storage at the elevated temperature and RH levels.

From the discussion above, it is apparent that it is rather onerous to gain a definitive correlation between the strategic design of dry powder inhaler formulation and the maintenance of optimal aerosolisation efficiency and protection against the detrimental influence of environmental conditions during processing, packaging and storage.

In light of all this, and in order to achieve a more comprehensive insight into the prospective influence of environmental conditions on the performance of dry powder inhaler formulations, a series of stability studies were undertaken. Both long term and accelerated stability studies were conducted on inhaler formulations exhibiting different types of lactose carriers. The lactose carriers employed were untreated air jet sieved lactose which was most significantly compared to the surface etched lactose. Furthermore, fine lactose particles were introduced to both commercial and surface etched lactose carriers, in an attempt to try to gain a better understanding of the role of fine particulates on maintaining aerosolisation efficiency of inhalation formulations upon storage at elevated conditions of relative humidity.

5.2 Materials

α-Lactose monohydrate (Lactochem® crystals) was supplied by Borculo Whey (Chester, UK). The lactose was vibrated through a nest of sieves to obtain a 63-90 µm sieve fraction, whish was used throughout the study. Air jet sieved lactose, which is 63-90 µm sieve fractioned commercial lactose that was further sieved by an air jet sieve. Sorbolac 400 lactose was supplied by Meggle (Wasserburg, Germany). Micronised salbutamol sulphate was supplied by Aventis Pharma (Cheshire, UK). HPLC grade Methanol was supplied by Fisher Chemicals (Loughborough, UK). Glacial acetic acid was supplied by BDH (Poole, UK). Ultra pure water was produced by reverse osmosis (MilliQ, Millipore, Molsheim, France).

5.3 Methods

General methods of each technique or apparatus used in this study are described in detail in Chapter 2.

5.3.1 Preparation and storage of powder formulations

Micronised salbutamol sulphate (median diameter, d_{50} 4.79 µm) was geometrically blended using a Whirlymixer (Fisons Scientific Equipment, Loughborough, UK) at a ratio of 67.5:1 w/w with untreated air jet sieved lactose (AJS), 5% surface etched lactose and both with the addition of 5%fine particle lactose (Sorbolac) within the percentage of total lactose considered in the blend. Blends containing fine particle lactose (Sorbolac) were prepared through forming an initial blend of coarse and fine lactose utilising the same method described in Chapter 2. This blend was then further mixed with micronised salbutamol sulphate to obtain the final blend.

Upon geometric mixing, blends were placed in a Turbula and mixed at 46 $rev.min^{-1}$ for 30 minutes. All blends were initially stored in a controlled environment of 44% RH for at least 24 hours before conducting content uniformity measurements.

Content uniformity was investigated by analysing 30.0 ± 2 mg samples (n=10) of each blend. Sample analysis was conducted using high performance liquid chromatography. Content uniformity of all blends gave a relative standard deviation of less than 5%. Hard gelatin capsules (Size 3) were filled with 30.0 ± 2 mg of each powder blend. Filled capsules were then divided into four batches for further *in vitro* performance investigations using the multi stage liquid impinger (MSLI).

One of batches was stored at ambient conditions, 44% RH at 25°C, prior to *in vitro* investigations. The constant humidity level was obtained by the use of a saturated salt solution of potassium carbonate which was placed in a tightly sealed container with the formulated blend (O'Brien, 1948).

Two batches were stored at 75% RH at 25°C for one and three months. The 75% RH level was obtained by the use of the saturated salt solution of sodium chloride placed in a tightly sealed container (O'Brien, 1948). The final batch was stored at 75% RH at 40°C for one month. These conditions were obtained by the use of the saturated salt solution of sodium chloride placed in a tightly sealed container, which was stored in a 40°C temperature controlled oven (O'Brien, 1948).

5.3.2 Drug content determination

Quantification of salbutamol sulphate content uniformity and *in-vitro* deposition was carried out by high performance liquid chromatography (HPLC). The HPLC system consisted of an AS950 intelligent sampler, PU-980 intelligent HPLC pump, 975 UV/VIS detector (all Jasco, Japan) and Spherisorb 15 cm, 5 µm ODS1 column (Waters, Milford, MA, USA).

The mobile phase used throughout the investigation was methanol/water (60:40) and acetic acid 0.1 % v/v. Settings were as follows: detection wavelength 276 nm; flow rate 1.25 $ml.min^{-1}$; pressure approximately 400 $Kg.m^{2}$; injection volume 100 µl; analysis time 4 min; approximate retention time 2 min.

Linearity was confirmed between 0.1 and 10 $\mu g.ml^{-1}$ ($R^2 = 0.99$). Lactose did not interfere with the salbutamol sulphate response. Sample injections were performed in duplicate using a bracket standard method containing standards prepared from separate stock solutions.

5.3.3 Dynamic vapour sorption

Moisture sorption profiles of the untreated air jet sieved lactose (AJS) and surface etched lactose were determined by dynamic vapour sorption (DVS) (DVS-1, Surface Measurement Systems, London, UK). Approximately 50 mg of powder sample was weighed into the sample pan of the DVS and exposed to a 0-90% RH cycle, at 10% RH increments. Equilibration moisture content, at each humidity, was determined with a dm/dt of 0.0002% min^{-1}.

5.3.4 In vitro aerosolisation studies

The pulmonary deposition of salbutamol sulphate was assessed by the use of a multi stage liquid impinger (MSLI) (Copley Instruments Ltd., Nottingham, UK). The MSLI apparatus consists of a throat, four classifying stages into which 20 ml of the HPLC mobile phase are introduced, and a fifth dismantled stage on top of which a fiber glass filter is placed. Upon achieving an air tight seal, single capsules containing the nominal dose of 400 ± 30 µg of salbutamol sulphate were actuated *via* a Cyclohaler® (Novartis, Surrey, UK) at 60 $L.min^{-1}$ for 5 seconds. The device is specifically fitted with a moulded rubber mouthpiece for attachment to the throat piece of the liquid impinger. The flow rate was adjusted using a rotary vein pump and solenoid-valve (Copley Scientific Ltd., Nottingham, UK) and calibrated using a reference flow meter. The pump was allowed to run for 4 seconds prior to and post solenoid-valve actuation, to allow the pump time to settle. The concentration of drug in each stage was determined by rinsing with mobile phase both the inner wall and the collection disc of the corresponding stage, followed by tilting and rotating the apparatus. The device, metal throat and the filter were also washed for drug extraction. The cut-off mass median aerodynamic diameters were 13.0, 6.8, 3.1 and 1.7 µm for stages 1, 2, 3 and 4, respectively, at 60 $L.min^{-1}$. The aerosolisation characteristics were described as: loaded dose (LD) which represents drug recovered from the device, mouthpiece, throat and all stages of the MSLI; emitted dose (ED) which represents drug emitted from the device into the mouthpiece, throat and all stages of the MSLI; fine particle dose (FPD) which represents drug deposited on stages 3, 4 and 5 (effective cut-off diameter ≤ 6.4 µm); fine particle fraction (FPF) which represents the percentage of FPD to LD.

5.3.5 Optical imaging of powder samples

Digital optical images of samples of powder blends were taken immediately after each formulation was emptied from representative capsules that were

stored for the specified time periods at the various environmental conditions investigated throughout the study.

5.4 Results and discussion

5.4.1 Physical characterisation

To achieve a comprehensive understanding of the influence of environmental storage conditions on formulation stability and performance, the deposition behaviour the DPI formulations and physical characterisation of the various lactose carriers was initially conducted. Lactose samples were characterised *via* their particle morphology, particle size and moisture sorption characteristics.

Representative scanning electron photomicrographs of air jet sieved lactose, surface etched lactose, air jet sieved lactose with fines and surface etched lactose with fines are shown in Figure 5.1. As previously discussed, the images clearly display the influence of the novel surface etching process on the surface roughness and the reduction in the level of intrinsic fines. Particle morphology investigations were also conducted on samples of lactose carriers, with and without added fines, upon storage at 40°C, 75% RH for one month as shown in Figure 5.2. On an individual particle level, there were no apparent differences for both air jet sieved and surface etched lactose samples with and without fines.

The cumulative undersize distribution of the four lactose carriers examined is graphically represented in Figure 5.3. The surface etching process resulted in removal of approximately 90% of fine lactose particles (quantified with regards to the percentage of particles exhibiting a diameter of less than 5 µm), when comparing the surface etched lactose with the untreated air jet sieved lactose. Surface etched lactose was found to have a median particle diameter of 99.54 µm with only 2.5% of particles having a diameter of less

than 5 µm. On the other hand, air jet sieved lactose with fines was found to have a median particle diameter of 15.85 µm with approximately 29% of particles having a diameter of less than 5 µm. In addition, surface etched lactose presented a volume median diameter (VMD) of about 112 µm, which was the highest among the lactose carriers examined in this study. Such findings indeed emphasise again on the dependence of apparent particle descriptors, and the fact that air jet sieving, for example, could not be considered as an efficient dependable method utilised for the removal of fine particulates. As a consequence, possible *inter* and *intra* batch variations may occur for such particles, thus giving rise to potential implications in terms of formulation performance.

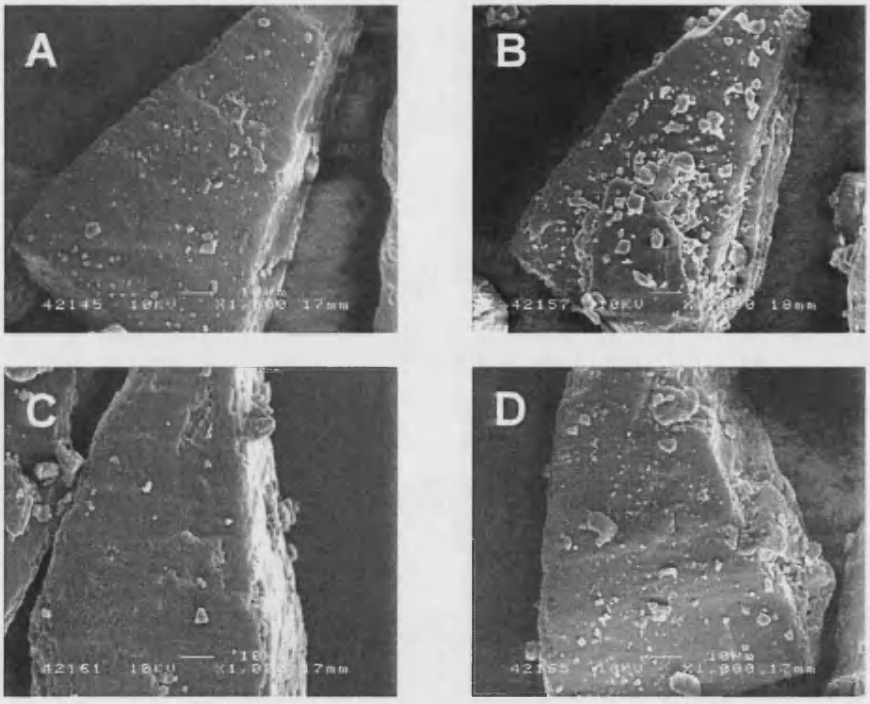

Figure 5.1. Representative SEM images of air jet sieved lactose (A), air jet sieved lactose with fines (B), surface etched lactose (C) and surface etched lactose with fines (D).

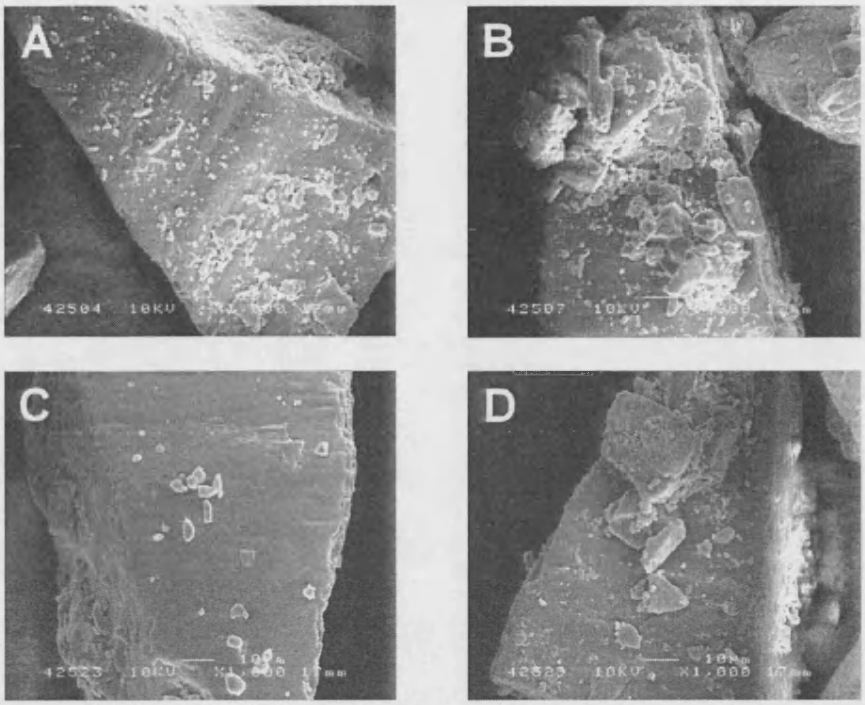

Figure 5.2. Representative SEM images of air jet sieved lactose (A), air jet sieved lactose with fines (B), surface etched lactose (C) and surface etched lactose with fines (D). The SEM images of lactose samples were taken following one month storage at 40°C, 75% RH.

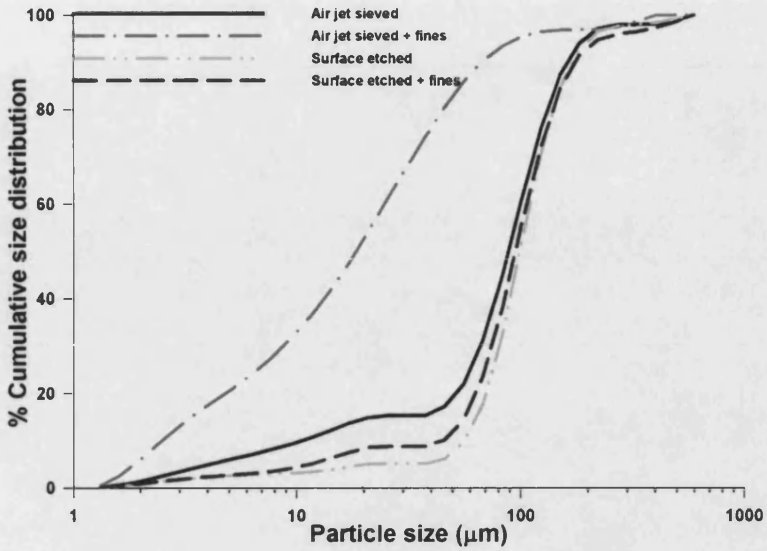

Figure 5.3. Cumulative undersize distribution graph of air jet sieved lactose, air jet sieved lactose with fines, surface etched lactose and surface etched lactose with fines.

Sorption isotherms, determined as percentage of dry mass, for untreated air jet sieved lactose and surface etched lactose samples are shown in Figure 5.4. All DVS experiments were conducted at 25ºC. The sorption isotherms clearly suggest that at low relative humidity levels (0-50% RH) both surface etched lactose and untreated air jet sieved lactose samples demonstrated a similar adsorption profile. However, above 50% RH increasing relative humidity led to a noticeable difference in their water adsorption behaviour. At 75% RH, for example, there was about 2.5 fold increase in the moisture sorption profile of air jet sieved lactose when compared to surface etched lactose. This difference increased with further increase in %RH. At 90% RH the difference was over 3 fold. A possible and logical explanation to such observations is that the surface of the untreated lactose particle may exhibit high surface free energy sites due to surface irregularities and/or increased sorption propensity at high levels of %RH associated with amorphous disorder related to the powder processing conditions of the lactose.

Meanwhile, the surface passifying effects of the temperature controlled surface etching process, may reduce the degree of reactive "hot spots" for moisture sorption, thereby leading to a decrease in the equilibration sorption of moisture at elevated conditions of relative humidity. This may be achieved by a possible reduction in the degree of high surface energy sites available for moisture condensation and the possible reduction in the degree of amorphous disorder, which is well-known to increase the degree of moisture sorption (Buckton *et al.*, 1988; Ward and Schultz, 1995).

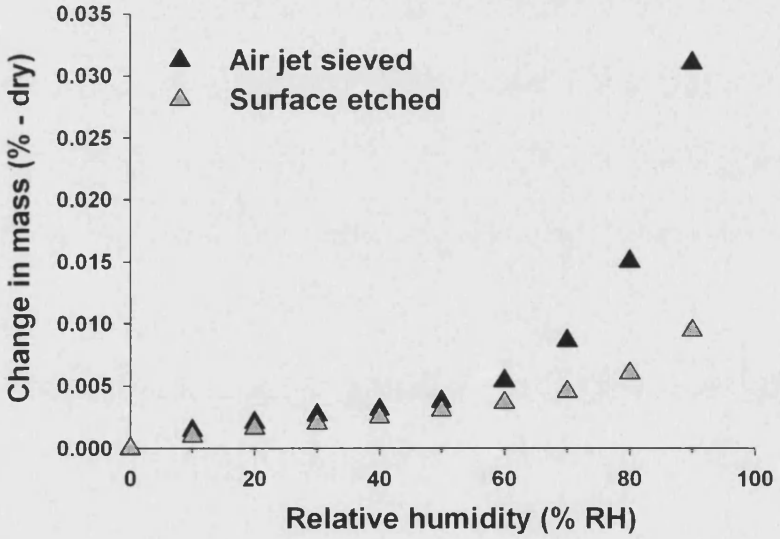

Figure 5.4. Effect of increasing relative humidity on the DVS isotherms of air jet sieved and surface etched α-lactose monohydrate samples.

5.4.2 In vitro aerosolisation studies

The fine particle delivery efficiency of salbutamol sulphate from the prepared blends of air jet sieved lactose, air jet sieved lactose with fines and surface etched lactose and surface etched lactose with fines as a function of varying accelerated stability conditions are shown in Figures 5.5 and 5.6,

respectively. The aerosolisation performance of the formulations, which were calculated in terms of fine particle dose (FPD) and fine particle fraction (FPF), are also tabulated in Tables 5.1 and 5.2, respectively. Figure 5.5 shows the comparison in FPF for the prepared formulations upon storage at 25°C, 75% RH for one and three months with respect to a control, which was conducted at ambient conditions (25°C, 44%RH). Figure 5.6 shows the comparison of FPF performance of the formulations upon storage at 40°C, 75% RH for a month, with respect to the control at ambient conditions.

When comparing the performance of the control blends, there is no significant difference in the aerosolisation efficiency of the model drug from air jet sieved lactose, air jet sieved lactose with fines and surface etched lactose blends ($P > 0.05$). However, a dramatic increase (approximately 90%) in the delivery performance of the drug from surface etched lactose was achieved upon the introduction of 5% fine lactose.

Interestingly, however, the fine particle delivery performance of the blends varied significantly upon storage at 25°C, 75% RH for one month. A significant decrease in the FPF performance was observed with both air jet sieved lactose ($p \leq 0.001$) and air jet sieved lactose with fines ($p \leq 0.05$) when compared to their control values at ambient conditions. On the contrary, there was no significant difference in FPF of drug delivered from surface etched lactose when compared to its control value upon storage at 25°C, 75% RH for one month. The most significant decrease in FPF of the drug ($p \leq 0.001$) was obtained from the surface etched lactose with fines blend, exhibiting a decrease in FPF of approximately 67% with respect to its control value. This significant drop in FPF of surface etched lactose with fines blend was not only seen in this particular storage condition, but also upon storage at 25°C, 75% RH for three months and upon storage at 40°C, 75%RH for a month. Fisher's pairwise comparison of the ANOVA data for surface etched lactose with fines found significant differences in FPF upon three months storage at 25°C, 75% RH ($p \leq 0.001$) with a 67% decrease in FPF (Figure 5.5), and also upon one month storage at 40°C, 75% RH ($p \leq 0.01$), with a 55% decrease in FPF (Figure 5.6).

In a similar manner to its behaviour at 25°C, 75% RH for one month, surface etched lactose showed no significant difference in delivery performance upon storing the formulation at 25°C, 75% RH for three months (Figure 5.5). Furthemore, no significant differences were observed upon storage at 40°C, 75% RH for one month (Figure 5.6). These data indicated that the delivery performance of the drug from surface etched lactose was to some extent maintained despite the exposure of the formulation to elevated conditions of temperature and relative humidity.

Meanwhile, air jet sieved lactose ($p \leq 0.001$) and air jet sieved lactose with fines ($p \leq 0.01$) blends exhibited a significant decrease in FPF upon storing the formulations at 25°C, 75% RH for three months with approximately 75% and 79% decrease in FPF, respectively. Interestingly, although the FPF of the air jet sieved lactose and air jet sieved lactose with fines decreased upon exposure to 40°C, 75% RH for one month, the decrease was not as significant as upon exposure to 25°C, 75% RH for three months (Figure 5.6).

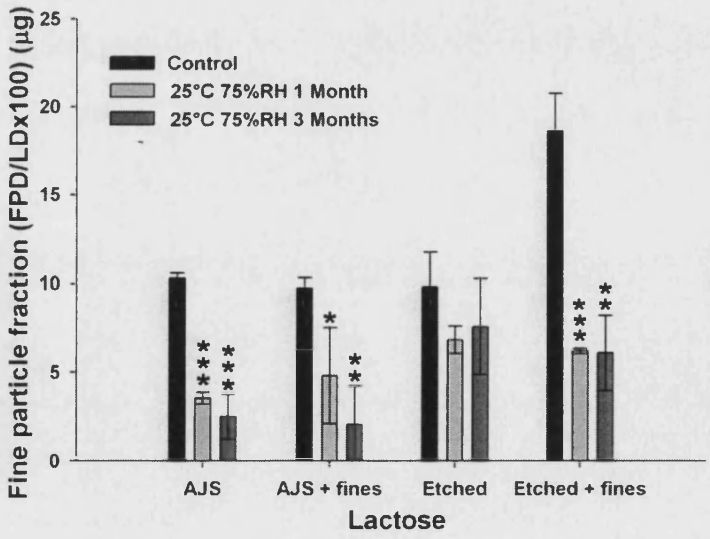

Figure 5.5. Effect of one and three months storage of powder blends at 25°C, 75% RH on fine particle fraction in comparison to control conditions. (*$p \leq$ 0.05, **$p \leq 0.01$, ***$p \leq 0.001$ confidence of variation by Fisher's pairwise comparisons).

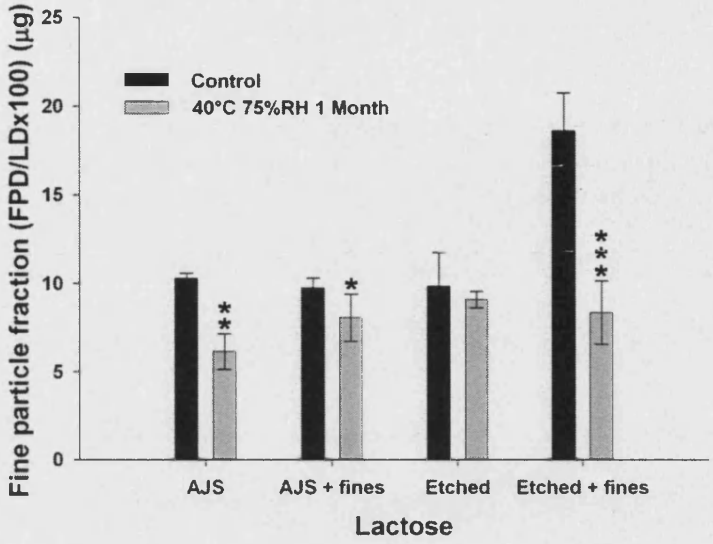

Figure 5.6. Effect of one month storage of powder blends at 40°C, 75% RH on fine particle fraction in comparison to control conditions. (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ confidence of variation by Fisher's pairwise comparisons).

More detailed graphical description of the percent to loaded dose of salbutamol sulphate deposited of all formulations on each stage of the MSLI apparatus for the control (25°C, 44% RH), 25°C, 75% RH for one month, 25°C, 75% RH for three months and 40°C, 75% RH for one month are shown in Figures 5.7, 5.8, 5.9 and 5.10, respectively. For control conditions, surface etched lactose with fines demonstrated the lowest deposition of drug at throat+stage1, and the highest deposition at stages 3 and 4. It is important to note, however, that at the throat+stage1 level, the deposition of drug from surface etched lactose formulation was less than that observed with air jet sieved lactose and air jet sieved lactose with fines formulations. When examining the deposition profile of salbutamol sulphate following storage under accelerated stability conditions (Figures 5.8, 5.9 and 5.10), surface etched lactose displayed the lowest percent drug deposited at the throat+stage1 level, implying that a higher percent of drug particulates reached the lower stages of the MSLI apparatus.

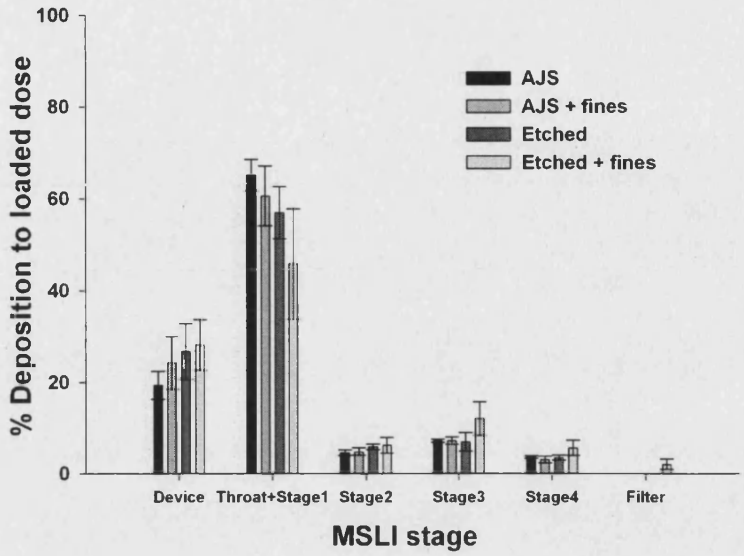

Figure 5.7. Percentage drug deposition to loaded dose on the MSLI stages at control conditions from the lactose formulations under examination.

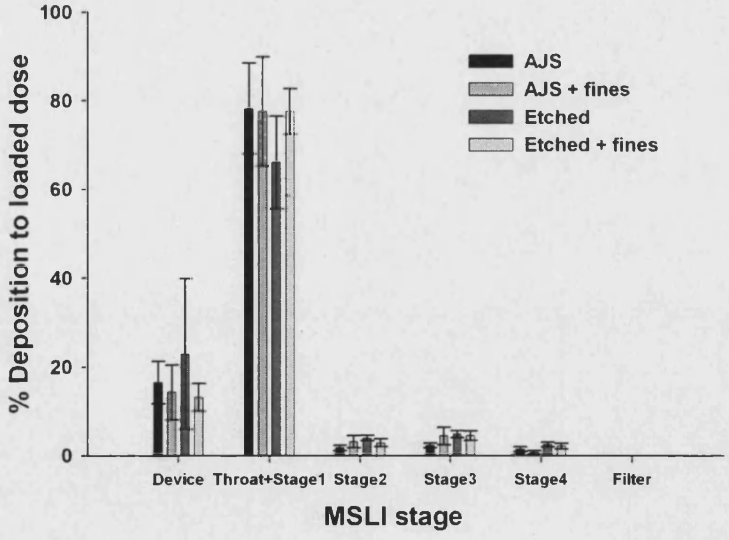

Figure 5.8. Effect of one month storage at 25°C, 75% relative humidity on the percentage drug deposition to loaded dose on the MSLI stages from the lactose formulations under examination.

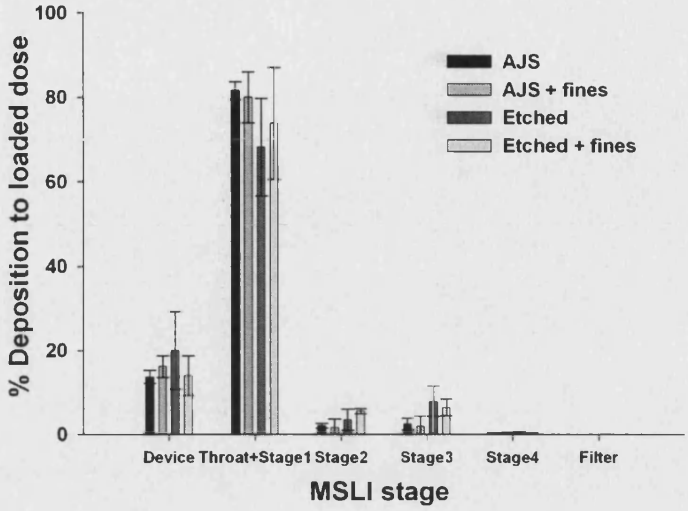

Figure 5.9. Effect of three months storage at 25°C, 75% relative humidity on the percentage drug deposition to loaded dose on the MSLI stages from the lactose formulations under examination.

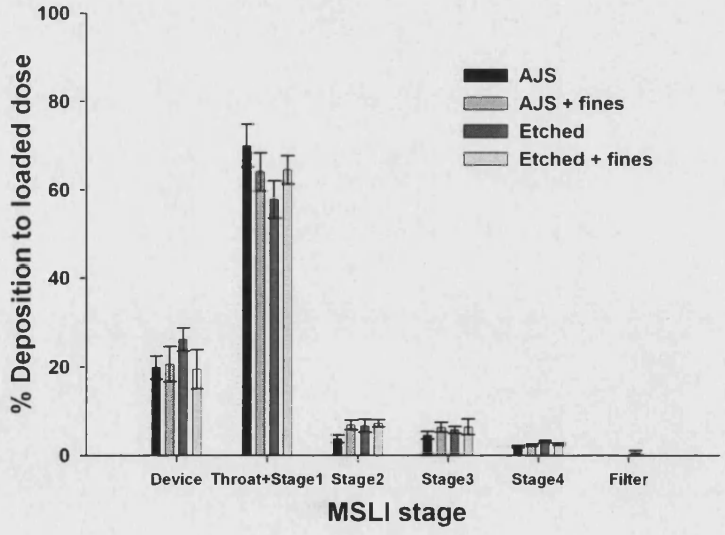

Figure 5.10. Effect of one month storage at 40°C, 75% relative humidity on the percentage drug deposition to loaded dose on the MSLI stages from the lactose formulations under examination.

Carrier	FPD (µg)			
	Control	25°C/75%RH 1M	25°C/75%RH 3M	40°C/75%RH 1M
Air jet sieved lactose (AJS)	44.7 ± 1.9	9.7 ± 0.8	7.6 ± 3.9	24.2 ± 3.9
AJS + fines	45.9 ± 1.3	15.5 ± 7.8	5.2 ± 5.6	34.4 ± 5.4
Surface etched lactose	37.0 ± 8.4	19.6 ± 2.5	20.7 ± 9.4	33.4 ± 3.0
Surface etched + fines	80.5 ± 21.9	19.9 ± 1.1	17.3 ± 5.1	34.8 ± 7.6

Table 5.1. Influence of environmental storage condition on fine particle dose of salbutamol sulphate (mean ± S.D. are shown; n=3).

Carrier	FPF (%)			
	Control	25°C/75%RH 1M	25°C/75%RH 3M	40°C/75%RH 1M
Air jet sieved lactose (AJS)	10.3 ± 0.3	3.5 ± 0.3	2.5 ± 1.3	6.1 ± 1.0
AJS + fines	9.7 ± 0.5	4.8 ± 2.7	2.0 ± 2.2	8.0 ± 1.3
Surface etched lactose	9.8 ± 1.9	6.8 ± 0.8	7.6 ± 2.7	9.1 ± 0.5
Surface etched + fines	18.6 ± 2.1	6.2 ± 0.1	6.1 ± 2.1	8.3 ± 1.8

Table 5.2. Influence of environmental storage condition on fine particle fraction of salbutamol sulphate (mean ± S.D. are shown; n=3).

To further probe the potential mechanism for the decrease in performance upon storage at conditions of elevated temperature and relative humidity, the potential for caking of the prepared formulations were examined *via* optical imaging. Optical images were taken immediately after releasing the powder blends from the capsules that were stored at the various storage conditions. Optical images of the blends released from capsules stored for one month at 25°C, 75% RH, three months at 25°C, 75% RH and one month at 40°C, 75% RH are shown in Figures 5.11, 5.12 and 5.13, respectively. In contrast to the microscopic analysis of individual particles with the SEM, macroscopic scale images of the powder formulation indicated the possible mechanism of failure of the carrier based formulations upon exposure to accelerated stability conditions. The optical images showed that for all environmental storage

conditions, air jet sieved lactose, air jet sieved lactose with fines and surface etched lactose with fines underwent considerable degree of powder clustering (agglomeration). However, such powder aggregation was not observed with the surface etched lactose particle blend. Such physical bonding and crystal agglomeration may be an indication of a considerable increase in inter-particle adhesion due to capillary interaction, arising from the condensation of the increasingly adsorbed water vapour layers onto the surface of crystals at high RH levels, as a consequence, promoting the formation of a capillary meniscus around the contact points of contiguous surfaces.

Another possible source of particle surface instability is thought to be the presence of amorphous domains which are liable to re-crystallisation at conditions of elevated RH levels (Briggner *et al.*, 1994; Buckton and Darcy, 1995) leading to solid crystal bridging.

Lactose crystals processed *via* the temperature controlled surface etching technique were likely to exhibit reduced amorphous content and passivated highly energetic surface sites. This maybe indicated by the decrease in the amount of water vapour adsorbed onto the surface at high RH levels. This led to no apparent powder agglomeration of the temperature controlled surface etched lactose formulations.

Another point to consider is that the addition of fine lactose particles may exacerbate the instability of carrier based formulations when subjected to environmental conditions of elevated temperature and relative humidity. This may be in part attributable to a potential increase in the overall surface area available for water vapour adsorption. Moreover, as a result of the high energy processing of fine carrier particulates, they are also more likely to show evidence of crystalline disorder, demonstrating a proportion of amorphous regions that would further enhance solid crystal bridging due to re-crystallisation at conditions of elevated temperature and humidity.

As previously discussed, while the introduction of a specified percentage of fine lactose particulates to surface etched lactose formulations demonstrated a striking increase in delivery performance at control conditions, such performance was markedly decreased following storage at all the tested environmental conditions. Thus, the enhanced delivery performance achieved upon the addition of fines could not be maintained as function of accelerated stability testing. A considerable 67% decrease in the aerosolisation efficiency was observed upon storing surface etched lactose with fines formulation at 25°C, 75% RH for one and three months. Moreover, a 55% drop in FPF was also noticeable when surface etched lactose with fines formulations was exposed to a condition of 40°C, 75% RH for one month. Moreover, surface etched lactose formulations showed a 30% decrease in FPF performance when stored for one month at 25°C, 75% RH, a 22% decrease in FPF performance when stored for three months at 25°C, 75% RH and finally a 7% decrease in FPF performance when stored for one month at 40°C, 75% RH. Such observations clearly indicate that the surface etched lactose formulation was indeed the least formulation to be affected by the exposure to the various environmental conditions in spite of the fact that it did not yield the highest FPF value upon aerosolisation at control conditions. The results obtained from this study emphasise the critical significance of conducting long-term and accelerated stability studies when considering the implementation of crucial modifications to a DPI formulation. Therefore, ensuring that enhanced performance at control conditions would be to some extent maintained in terms of powder homogeneity and reproducibility even upon exposure to rather extreme environmental conditions.

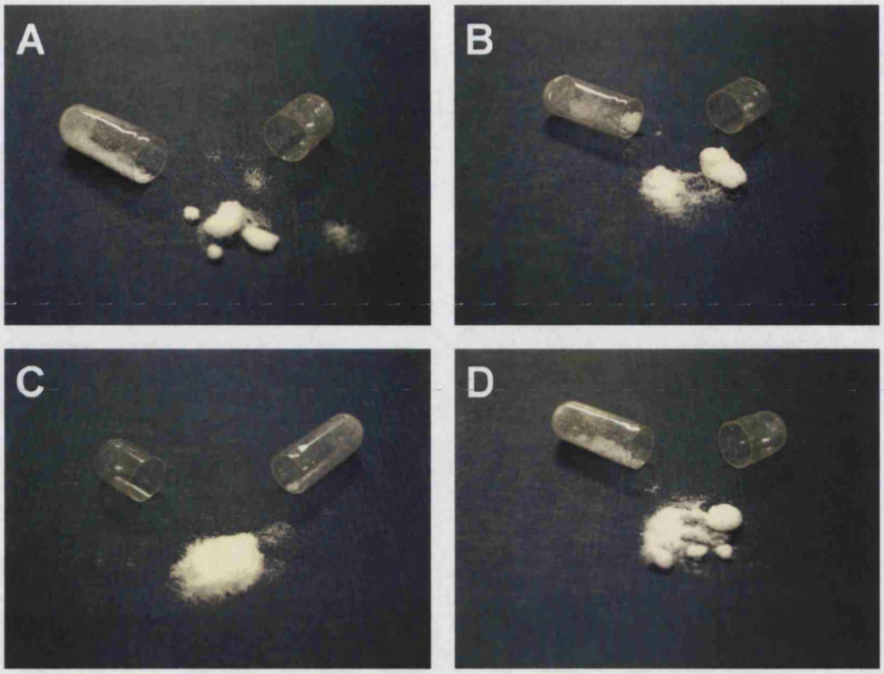

Figure 5.11. Optical images of air jet sieved lactose (A), air jet sieved lactose with fines (B), surface etched lactose (C) and surface etched lactose with fines (D). Images indicate the influence of increased RH on the behaviour of powders released from capsules stored for one month at 25ºC, 75% RH.

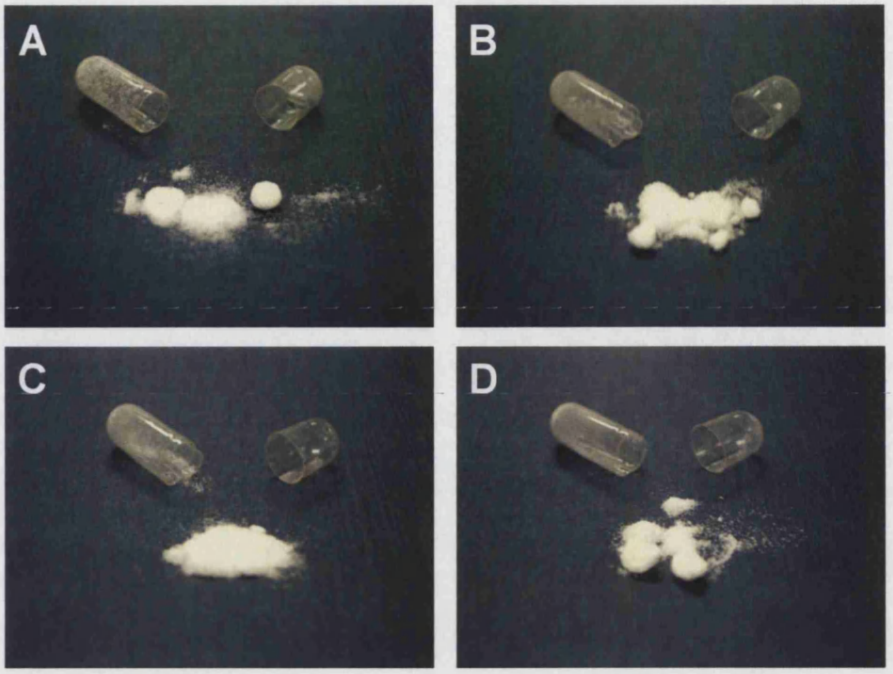

Figure 5.12. Optical images of air jet sieved lactose (A), air jet sieved lactose with fines (B), surface etched lactose (C) and surface etched lactose with fines (D). Images indicate the influence of increased RH on the behaviour of powders released from capsules stored for three months at 25ºC, 75% RH.

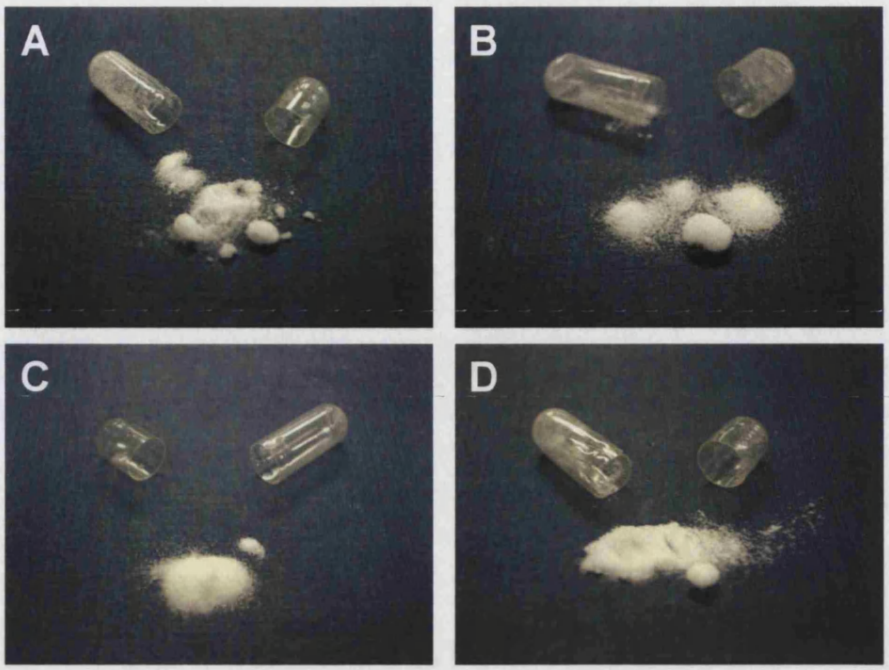

Figure 5.13. Optical images of air jet sieved lactose (A), air jet sieved lactose with fines (B), surface etched lactose (C) and surface etched lactose with fines (D). Images indicate the influence of increased temperature and RH on the behaviour of powders released from capsules stored for one month at 40ºC, 75% RH.

As previously discussed, the interplay of a number of factors may possibly give rise to an increase in the interaction force between particles when subjected to elevated temperature and RH levels. Such crucial elements could be the presence of thermodynamically unstable sites that are usually in the form of interchangeable crystalline/amorphous domains. Moreover, the inconsistency in surface roughness of untreated mechanically processed carrier particles, may be the source for increased water sorption leading to increased particle-particle interactions.

In view of all this, the novel temperature controlled surface etching process of commercial grade α-lactose monohydrate particles may indeed be perceived as a valuable approach towards the surface conditioning of the rather unstable industrially processed carrier particles. Such surface conditioning

involves a possible elimination of particle irregularities, alleviation of surface amorphous regions, optimisation of surface roughness. Thus, allowing the production of carriers which exhibit improved batch to batch consistency. Further, the conditioning of the lactose particle surface has been shown to possibly enhance the physicochemical stability of dry powder inhaler formulations at extreme environmental conditions.

Another key element to take into consideration, is the possible physical instability arising from the introduction of fine particulates. Such instability was markedly profound particularly following the exposure of fines-containing formulations to the intense environmental conditions.

The possible role that fine carrier particulates play in issues related to the stability and possible loss in performance of a carrier based dry powder inhaler formulation has not been fully understood. Investigations relating to the use of fines, has mainly been focussed on the fundamental understanding of the influence of fines on the increased aerosolisation performance of dry powder inhaler formulations upon processing. A number of theories have been employed in order to explore the exact mechanisms by which they are thought to enhance the aerosolisation performance. A logical explanation that could be predicted is the they preferentially occupy highly energetic 'active site', thus leaving the low adhesion 'active sites' more available from drug particulates. It is without doubt, however, that one of the most significant outcomes of this part of the study, is that it highlighted the potential 'negative' influence of fine carrier particulates in the long term stability of the formulation. This study has clearly emphasised the need to understand long term stability issues and to combine the influence of all interacting factors under a broad fundamental insight of the means to achieve and maintain aerosolisation performance.

5.5 Conclusions

Variations in temperature and RH levels of the surrounding environment of a carrier based DPI formulation was found to markedly influence its pulmonary delivery performance. Significant variations in the fine particle dose and fine particle fraction were obtained as a function of storage at various atmospheric conditions. The study indicated that the use of surface etched α-lactose monohydrate particles maintained efficient pulmonary drug delivery following exposure to elevated environmental conditions. The study suggested that surface roughness variations of carrier particles may strongly be a possible source of *inter* and *intra* batch inconsistency therefore more profound physicochemical instability of formulations. Additionally, the stability of formulations demonstrating a higher percentage of fine carrier particulates was significantly threatened, suggesting the significance for further studies to be conducted as to the assessment of the role of intrinsic and extrinsic fines for long-term stability of carrier based dry powder inhaler formulations.

Chapter 6

Conclusions

6.1 Introduction

The efficient development of a dry powder inhalation system in yielding an effective pulmonary delivery is critically dependent on the interplay of a myriad of crucial determinants. However, the key factors affecting de-agglomeration and aerosolisation of respirable particles may possibly be reflected by two main competing physical aspects. These are the inter-particulate forces acting between contiguous particles surfaces and the long term physicochemical stability of DPI formulations upon storing and handling at various environmental conditions of temperature and humidity. It is also significant to note however, that the effect of these two elements relates to the aerodynamic forces created by the inspiratory air flow applied by the patient, promoting the fluidisation and de-agglomeration of the respirable particles. Thus, it is logical that a prediction and to some extent a control of the physicochemical determinants is thought to be of uppermost significance in the attempt of gaining a more comprehensive understanding of the paradigms and fundamentals of the behaviour of carrier based dry powder inhaler formulations.

6.2 Summary

The primary aim of this study was to develop a novel surface etching method that would enable controlled modification of the nanometer scale surface characteristics of excipient particles. An apparent and direct correlation was further established between the qualitative characterisation of the surface morphology of etched lactose excipient particles and the quantitative *in vitro* deposition profile of the respirable fraction of the model drug salbutamol sulphate that was employed throughout the study.

Preliminary findings suggested the existence of an apparent relationship between the surface topography of excipient particles in terms of roughness and the aerosolisation performance of salbutamol sulphate. The efficient delivery of the respirable fraction of the drug was found to be significantly improved upon decreasing the surface roughness of lactose carrier particles. Nonetheless, the study also clearly indicated a proposed manipulation of drug-carrier inter-particulate interactions as a function of increased degree of surface etching. That is to say, optimisation of surface etching is an essential requirement for maintaining most advantageous cohesive/adhesive characteristics that would ensure both reproducibility and long term stability of the DPI formulation.

Moreover, one of the most significant outcomes of the novel temperature controlled surface etching process besides modifying the surface roughness of excipient particles, is the possible attainment of *inter* and *intra* batch consistency. Such cross-batch equivalency may possibly be regarded as one of the imperative determinants influencing formulation performance. Such equivalency resides in the concept of obtaining consistent and dependable removal of intrinsic fine particle content and controllable modification of surface roughness.

Surface etched lactose particles were found to relatively improve the respirable fraction of tested DPI formulations for low drug doses. These findings suggested that surface etching of lactose particles may have

resulted in a considerable decrease of 'active sites', thus reducing the fraction of the dose utilised solely to passify the 'active sites'. As a consequence, the remaining unbound fraction, which is more prone to adhering to the lower energy 'passive sites' may increase the delivery efficiency. With regards to surface etched lactose particles, the saturation level of active sites maybe reached with a significantly lower dose in comparison to untreated commercial grade and air jet sieved lactose particles.

A parallel investigation was also carried out as part of an attempt to study the influence of environmental conditions on dry powder formulation delivery characteristics. A series of both long term and accelerated stability studies were performed. Dry powder formulations exhibiting surface etched lactose and untreated air jet sieved lactose were subjected to various storage conditions. Furthermore, a defined percent of fine lactose carrier particulates was introduced to both surface etched and untreated air jet sieved lactose particles, and such formulations were also subjected to the same storage conditions. Initial investigations conducted at control conditions indicated an apparent and noticeable increase in delivery performance which was clearly demonstrated by surface etched lactose upon the introduction of fine carrier particulates. This was not the case, however, following the storage of tested formulations at the various environmental conditions. The highest drop in fine particle delivery performance was obtained from the surface etched lactose with fines blend at all tested environmental conditions. Air jet sieved lactose and air jet sieved lactose with fines also exhibited evident variations in their delivery performance upon exposure to rather sever conditions of elevated temperature and relative humidity (RH) levels. Conversely however, the delivery performance of the drug from surface etched lactose formulations was significantly more stable despite the exposure of formulations to the elevated temperature and RH levels at all the designated environmental storage conditions. Such findings emphasise on the surface instability that was introduced upon the addition of fine carrier particulates. The study also highlighted that more profound physicochemical instability is strongly related

to the surface roughness variations and particle irregularities that are perceived as cross-batch inconsistencies. Moreover, the study also indicated that optimal environmental conditions during processing, handling and storage of DPI formulations are indeed a necessity towards achieving the desired delicate balance of inter-particulate interactions.

Overall one can predict that the novel temperature controlled surface etching process may indeed offer a plausible approach towards alleviating unfavourable shortcomings that may possibly be related to various excipient batch discrepancies. This in turn would possibly enhance and to some extent maintain the efficiency of pulmonary drug delivery of dry powder inhaler formulations, thus establishing the solid basis for the future perception of further improvements that are yet to be achieved in the field of dry powder inhalation pharmaceuticals.

6.3 Suggested future work

As a result of this research study the use of a novel temperature controlled surface etching technique for modifying the surface physicochemical properties of commercial grade lactose has been shown to provide a better methodology for further investigations to be carried out with the goal of presenting a more comprehensive understanding of the quantification of surface characteristics of excipient particles and their direct correlation to inter-particulate forces governing the behaviour of aerosolised powders.

Notwithstanding, the novel temperature controlled surface etching process has been so far only applied on α-lactose monohydrate carrier particles. Further investigations into its applicability to other various excipient particles may indeed be taken into consideration. Another interesting field of study that might be of significant importance is the possibility of subjecting fine carrier particulates to the surface etching process, therefore investigating the

controlled adjustment of the critical etching parameters in order to render it applicable to lower particle size distributions.

Extended effort may also be undertaken in order to gain a more valuable insight into the ambiguous role of fine carrier particulates on aerosolisation behaviour. Moreover, such investigations are more preferably to be conducted concomitantly with more extensive long term stability studies. For example, surface etched fine carrier particulates might offer a more plausible solution for the attainment of long term efficiency and reproducibility of powder formulations despite the various intense storage conditions, all of which are yet to be explored.

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